HANDBOOK

OF

PRACTICAL BOTANY

FOR THE BOTANICAL LABORATORY AND PRIVATE STUDENT

BY

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TRANSLATED AND EDITED FROM THE GERMAN
WITH MANY ADDITIONAL NOTES

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WITH 166 ORIGINAL AND A FEW ADDITIONAL ILLUSTRATIONS

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PREFACE TO THE FIRST GERMAN EDITION.

THIS book is intended chiefly for those who, without desiring to become botanists by profession, wish nevertheless to become acquainted with the elements of scientific structural botany. It will likewise introduce the beginner to the various methods of microscopical manipulation.

The study of vegetable structure is especially favourable as an initiation into the use of the microscope; and any one whose future career will require command over this instrument should commence with the study under the microscope of vegetable anatomy.

The manual is divided into thirty-two chapters, each of which is intended to provide materials for several hours' practical work in the laboratory. The earlier chapters are easy, and the difficulties to be encountered increase almost continuously up to the last chapter. The first chapter assumes on the part of the worker entire ignorance as to the use of his instruments, but nevertheless assumes the possession of some general botanical knowledge. With this elementary preparation the beginner ought to be able, by the diligent use of this book alone, to acquire a tolerably broad knowledge both of vegetable structure and of the methods of microscopical work.

The objects for study have been so selected that most can be obtained with comparative ease. In many places I have recommended the use of plants preserved in alcohol, as the worker is thus rendered independent of the time of year. As, however, the objects may need to be collected even months before being used, the student onght carefully to consult a special list of plants or portions of plants needed for his work, and which ought to be collected at some given time

or condition. Not infrequently the objects need to undergo, in order to make them fit for use, some preliminary preparation which may take several hours or even a day. The student ought therefore to take cognisance of a lesson a sufficiently long time before commencing work.

The list of necessary reagents will be found at the end of the book. These reagents should be ready before beginning work. The method of preparation of special reagents for histological work is also given in this list. Many of these it is preferable to obtain ready-made from the firm mentioned at the head of the list.

The explanations and illustrations of the use of the instruments and reagents are scattered in the text; but the general index is made so far complete as to enable the student easily to refer to any explanations which may be necessary.

I have given especial care to the methods of study of the Bacteria; with tbe preparation which this book gives, the student will be capable of following out any deeper researches into this subject, as well as of realising their practical application.

All the figures in this work have been drawn by myself from Nature. Almost all of the facts given in the text, even those which were well known, have been submitted to careful control.

At the close of each chapter are given some bibliographical notes, which show the student the fountain-head whence fuller information can be obtained.

ED. STRASBURGER.

PREFACE TO THE FIRST ENGLISH EDITION.

ALTHOUGH the last two or three years have produced at least as many works on Practical Botany for laboratory purposes, no apology is needed for reproducing from the German one which has no counterpart in the English language, and which has the advantage of being written by one of the greatest living masters of microscopical observation.

Although Professor Strasburger has revolutionised the science of Botany in more than one direction, no work of his has as yet come before an English public in its own tongue; bnt it is perhaps not unfitting that the Author's first introduction to the English-reading student should be in the *rôle* of teacher of those arts of manipulation and observation by the exercise of which his own fame has been gained.

This edition has the advantage of revision and of numerous additional notes by the Author; some portions have been well-nigh rewritten. To these I have ventured to add notes of my own, intended to either simplify or amplify the description, or to enable the material selected by the Author to be replaced by some other, probably more readily obtainable. These additions have been either inserted as footnotes, or, where intercalated in the text, are usually inserted between square brackets [ ]. The Introduction I have, with the consent of the Author, nearly rewritten, in order to make it more suited to the English student; similarly with a few other isolated paragraphs. A few additional illustrations I have been enabled to add, through the courtesy of the Publishers; descriptions of, and references to, these are likewise enclosed in brackets. To make the book more

convenient in use, I have given at the head of each chapter (task, or lesson, in the original) a list of the objects required for study in that chapter. I regret that I did not add to these lists any special reagents which might be required for use; possibly a future edition may give opportunity for this.

I have considerably enlarged the scope of Appendices II. and III., and have added two new Appendices I. and IV., which I hope may be useful to the student. Throughout the work I have likewise added the common English names (if any) of the plants referred to.

The student will probably not be able to carry out all *the investigations constituting a chapter at the same time.* A careful note should be made of any which are thus postponed, so that they may be taken up in due season. It is not unlikely that some may not come at all within the range of the student's observation; for these examples the book must be looked upon in the light of a text-book.

Tbe student is earnestly urged to study from the beginning the Author's methods of work. These are especially noteworthy when he comes, perhaps incidentally, to correlate structure with function. The interdependence of these two factors in the plant's life history is the great underlying principle of modern botanical teaching, and the student cannot too soon begin to exercise his thoughts in this direction, resting assured that his methods are right even though his results may for the time being prove to be erroneous.

As to translation, no one can feel so fully as myself its many and serious defects. I can only plead that the work was executed at a time of serious pressure, and, although circumstances have delayed the issue of the book, the manuscript was out of my hands, and therefore only subject to such limited correction as proof-sheets would allow.

W. H.

BIRMINGHAM, *September*, 1886.

PREFACE TO THE SECOND ENGLISH EDITION.

ALTHOUGH anything like a complete revision of the text has been impracticable, I have been enabled, through the liberality of the Publishers, to make several considerable and important additions both to the text and figures (mainly derived from the Second Edition of the *Botanisches Praktikum*), and the chapters upon "Vascular Bundles" and "Bacteria" have been to some extent rewritten. . . . I have likewise made a few verbal and other corrections, and, after much hesitation, have eliminated the square brackets wherever this has been feasible.

I have most heartily to thank many correspondents here, in America, and elsewhere, for numerous notes, memoranda and criticisms. Although I may not at present have been able to make use of them, these suggestions will not be lost sight of; and, even should they never be used, I am none the less grateful for the kindly spirit which has prompted their transmission.

W. H.

1st *August*, 1889.

PREFACE TO THE FIFTH ENGLISH EDITION.

THE present edition of this work has been rewritten almost throughout. In doing this full use has been made of the third edition of the Author's *Das Botanische Praktikum*, which appeared in 1897. A number of new figures have been introduced, the most important of which are a series dealing with the histology of *Tilia* and of *Pinus*.

As far as possible I have endeavoured to avoid contentious matter, believing such to be out of place in a book of this kind; but it must be borne in mind that the "facts" of a science such as Botany are to a large extent inferences or interpretations, so that sources of contention cannot be entirely eliminated, and new ones are at any time liable to arise. It has been a matter of difficulty to determine to what extent certain varying interpretations should be recognised, and in some cases, in endeavouring to avoid possible confusion in the mind of the student, I have perhaps gone astray, as, for example, in retaining the old term "vessels" for many structures the tracheïdal character of which is now generally accepted.

As the practical advantages appeared to warrant it, the numerous notes derived from my own laboratory practice, and other sources, which had been introduced into the earlier editions, or are new to this, have now as a rule been fully incorporated with the text. The curious in such matters can doubtless disinter them by a comparison of the first English edition (in which all such additions were in square brackets), and of the German third edition, with the present issue.

After careful consideration I have omitted the bibliographical notes which were appended to the chapters. As the

references were very largely to German sources, it follows that any who were capable of making use of them would be also capable of referring to them as set out at length in the German original.

I have to thank my friend and former pupil, Dr. A. H. Reginald Buller, for assistance in simplifying some of the formulae, and testing the resulting reactions.

W. H.

UNIVERSITY OF BIRMINGHAM,
July, 1900.

PREFACE TO THE SIXTH ENGLISH EDITION.

A CONSIDERABLE amount of textual revision has been found possible without altering the pagination; new figures have replaced a few which were obsolete, and some others are added. The chapter on "Cell and Nuclear Division," however, has been in part rewritten; and I have ventured to rearrange the material in such way as to illustrate grades in cytological technique.

Many botanical laboratory manuals have been published since this first appeared in English, twenty-one years ago; *but it may still help for a while to guide in morphological* method the hand and eye of successive generations of botanical students.

W. H.

UNIVERSITY OF BIRMINGHAM,
December, 1907.

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INTRODUCTION.

STUDENTS who work in properly equipped botanical laboratories will find therein the needful instruments. Those, however, who use this book at home, as an introduction to the microscopical study of plants, as well as those who, apart from these conditions, wish to become possessed of instruments suitable for such work, may perhaps find in the following pages some aid to their choice.

Starting with the assumption that the instrument is desired for the sake of the work that can be done with it, rather than for its own sake as a beautiful piece of optical and mechanical workmanship, it is well to strongly urge the intending purchaser to buy an instrument constructed upon what is known as the "Continental model". The microscopes built on the so-called "English model" are more massive and complicated in their construction than is really necessary for student purposes, and the object on the stage is usually moved about by means of a mechanical arrangement of screws, where, for, ordinary purposes, the fingers had better be used. Further, though the diameter of the body of the English microscope may be an advantage, its length is doubtfully so, and renders the erect position of the instrument in working, which is for most purposes far the best (though a joint permitting inclination is highly desirable), almost impossible. The distance of the stage from the eye renders delicate working with the fingers a matter of greater difficulty; for it is notorious that the nearer the fingers are to the eyes, within certain limits, the more delicately their movements can be controlled. Most, or all, of the great English and American makers, such as the well-known firms of Ross, Ltd.; Powell & Lealand; R. & J. Beck, Ltd.;

Bausch & Lomb, and Zentmayer have recognised the need for instruments of more compact form, simpler construction, and lower price, and, under one name or another, have brought out instruments on the lines (many of their faults included) of great Continental makers, such as Zeiss, Leitz, Seibert, Reichert, Hartnack, etc., whose microscopes are to be found by the thousand in the great laboratories of the European Universities.

Putting upon one side, then, alike the more elaborate and expensive instruments of microscope manufacturers, and those in which everything is sacrificed to cheapness, microscopes suited for student use may be roughly placed in two groups; the one containing comparatively simple instruments, a reasonably good microscope reduced, so to speak, to its simplest terms, in which the apparatus consists only of a means for steadily supporting and illuminating an object to be examined, and magnifying it with accuracy to a moderate extent; and the other group comprising instruments of a higher quality, to which accessory apparatus for illuminating or examining are, or can be, attached, and the magnifying capacity of which is of a higher degree of excellence. The first of these groups contains instruments which are quite suited to the greater part of the microscopical work detailed in this book. But should the student have ambitions, and not merely wish to study those few parts of this book which are comparatively inaccessible with the simpler instruments, but be desirous of providing him or herself with a microscope which may hereafter be used as a means to wider study, or even, may be, for the broadening of knowledge by new investigations, then a microscope selected from the second category becomes a necessity. It does not follow that the whole of the ultimate expense must be incurred at once, but it is essential that the microscope stand which is purchased shall be of a kind to which subsidiary apparatus can be fitted, and which will provide a suitable support for higher magnifying powers than those which may in the first instance be purchased.

The microscopes provided for early student use in botanical laboratories belong to the former of these groups, and will probably be found to have a magnifying capacity up to 400

or 450 diameters. Such microscopes are manufactured by very many makers; but as the narrow limits of this introduction compel rigid selection, our descriptions will be confined to a few representative forms of (price taken into consideration) good quality, and capable of sufficiently satisfactory work as judged from the beginner's standpoint. The price of each is affixed.[1]

R. & J. Beck, Limited, 68 Cornhill, London, E.C.

1. The "*London*" *Microscope*, *No.* 1125, with tube substage. Stand with horseshoe foot, and jointed pillar; spiral rack-and-pinion coarse and micrometer screw fine adjustments; one eye-piece, ⅔ and ⅙ (or ⅛) inch objectives; iris diaphragm; vulcanite stage; in mahogany case, £5 12s. 6d.; or with double nose-piece, £6 1s. 6d. This instrument is figured in page 2, and fully described in the text.
2. The "*London*" *Microscope*. *No.* 1153. An instrument of the same general design as the preceding, but of larger dimensions, and more fully fitted for the subsequent addition of accessories. With two eye-pieces, ⅔ and ⅙ (or ⅛) inch objectives, double nose-piece, and Abbé condenser with iris diaphragm, £10 1s.

Ross, Limited, 111 New Bond Street, London, W.

No. 2 "*Standard*" *Microscope* complete for senior, medical and biological work, including stand with jointed pillar, horseshoe foot, screw coarse and fine adjustments, one eye-piece, ⅔ and ⅙ inch objectives, double nose-piece, plain substage ring fitted with iris diaphragm; in mahogany case. Magnification, 50 to 500, £10 15s. If with a focussing screw-action swing-out substage, £11 10s.

James Swift & Son, University Optical Works, 81 Tottenham Court Road, London, W., have an exceptionally high reputation as mechanicians, and their microscope stands have shown more independent and original thought than those of perhaps any other maker.

1. *Histological and Physiological Microscope*, No. 16.—Body swung upon a specially designed tripod stand of unusual stability, with diagonal rack-and-pinion coarse and isolated micrometer screw fine adjustments, large stage, one eye-piece, ⅔ and ⅙ pan-aplanatic objectives, and iris diaphragm; in case, £6 15s. Accessories, double nose-piece and extra eye-piece, 15s., Abbé substage condenser, 10s.

[1] The subjoined memoranda of microscopes are not intended to give complete specifications, or to obviate the necessity of the personal study of microscope catalogues, or appeal for skilled assistance if accessible. They are to be looked upon as suggestions as to where to look and what to look for. All the microscopes mentioned are, however, of sound and reliable quality.

[2] It may be noted that nearly all objectives, British and foreign, are now made with screws of uniform diameter (the English Microscopical Society's standard screw), and therefore are mutually interchangeable. It must, however, be noted that objectives made for a short-bodied ("Continental") stand are not suited for use with a long-bodied ("English") stand, and vice versa.

W. Watson & Sons, 313 High Holborn, London, W.C.

1. "*Praxis*" *Microscope*.—Stand with horseshoe foot and jointed pillar; rack-and-pinion coarse and micrometer screw fine adjustments, one eye-piece, 1 inch and either ⅔ or ⅙ objectives; in mahogany case, £5 15s. Further eye-pieces and objectives as required, and substage fittings can be attached.

2. "*Fram*" *Microscope*, *F.* 149.—Body swung upon tripod stand of well-arranged form, with jointed pillar and with horseshoe stage covered with ebonite, diagonal rack-and-pinion coarse and micrometer screw fine adjustments, two eye-pieces, ⅙ inch objective and choice of 1 inch, ½ inch, or ⅜ inch; Abbé substage illuminator, with iris diaphragm; in mahogany case, £8 5s. Extras, double nose-piece, 10s. 6d. or 15s.

James Parkes & Son, Vesey Street, Birmingham, manufacture a series of inexpensive students' microscopes of simple but sound construction, suited for elementary histological work.

1. "*Worker*" *Microscope*, with body slung on a tripod foot, fine adjustment of hardened steel and gun metal, working in solid machine-planed box, and coarse adjustment by sliding tube in cloth-lined collar, one eye-piece, 1 inch and ⅙ objectives, in mahogany case, £3 15s.; or the same with diagonal rackwork coarse adjustment, £4 10s.
2. *The* "*Century*" *Microscope*, with body slung on tripod foot, large stage, diagonal rackwork coarse and improved micrometer screw fine adjustment (this latter placed in a new position to ease strain upon it), one eye-piece, ⅔ and ⅙ objectives, in case, £5 10s. Accessories are: Swinging substage, with screw adjustment, £1; "Abbé" condenser, with iris diaphragm, £1.

Of Continental makers, while noting the name of Zeiss, Jena (English office, 29 Margaret Street, Regent Street, London, W.), world-renowned for the quality of his optical appliances, I think best to limit the attention of the student to a German firm which in the last twenty years has won for itself a repute of the highest kind for fine and reliable mechanical and optical workmanship, combined with moderate price, *viz.*:—

E. Leitz, Wetzlar (London Office: 9-15 Oxford Street, W.).

1. *Students' Microscope*, *II. B*, with body jointed to tripod foot, rackwork coarse and micrometer screw fine adjustments, two eye-pieces, ⅔ and ⅙ objectives, magnifying from 60-525 diameters, double nose-piece and worm screw, to which a substage condenser can be subsequently added, £6 5s.

2. *Students' Microscope, II. A*, is figured on p. 8 and described in the text. With the appliances there mentioned, and including objectives 3 and 7 and eye-pieces 0, II. and IV., giving a range of magnification from 46 to 625, price £9 15s.[1]

Most, or all, of the above makers, English and foreign, manufacture microscope stands of cheaper quality than the above; it must, however, be borne in mind that accurate observation needs an instrument which is capable of it, and while there is, and ought to be, every desire to keep the cost within reasonable limits, true economy does not consist in purchasing an instrument which may be a constant source of dissatisfaction, and may have to be discarded directly the student emerges from swaddling clothes. Preferably, therefore, the stand which is purchased should be adapted to the receipt of optical apparatus other than the mere eye-piece and objective; should have a jointed back, and be thoroughly steady in any position; the adjustment should be easy and true, and if the body or draw-tube is twisted, any object observed should not be thrown out of centre; the mirror should be plane and concave, and should have a long, jointed arm; and the stage should be constructed for the reception of a substage condenser, and should be provided with an iris diaphragm. Still more essential is it that the special optical parts, the eye-pieces and objectives, should be good. They should let through the largest possible amount of light (the diaphragm will easily control its quantity, if needed), and there should be absence of colour, both round the exterior of the field of view, and round any object, or particles of dust, in focus. The field should be *flat*, so that a small object moved from one part to another alters neither in distinctness, form, nor size. The high power objectives should have a fair working distance from the object, or the thickness of the cover-glass, to be hereafter noted, may become a matter of great importance. Lastly, a double nose-piece of accurate construction should be provided, and the objectives should have their settings so graded that in changing from

[1] I (Ed.) have a number of these instruments in use in my own laboratory and think very highly of them.

one to another the object is retained approximately in focus.[1]

In all these points, except perhaps accurate centring, the stands of good English makers probably equal, price for price, the foreign stands; while, on the contrary, price for price, the eye-pieces and objectives of continental makers usually are far superior to those made by the English opticians—a superiority probably due solely to the more trained skill and more patient accuracy of the workmen.

All of the work in this book, with perhaps the exception of Chapters XXI. and XXXII., can be performed with the the aid of objectives up to $\frac{1}{8}$; but the student who has gained some experience will probably wish to add to his microscope one or more stronger objectives, in order to increase the range and delicacy of his work. Increased magnification can be obtained by increased power either of eye-piece or of object-glass. All the objectives we have heretofore noted are what are called "dry" systems, since they are used for work in a dry state, and a layer of air separates the objective from the object. "Dry" objectives of high power are subject to great disadvantage from the serious loss of light their use involves. The light, in passing from the mirror to the objective, passes in the first place through air, then the object-slide, next the object and the medium in which the object is mounted, which may be glycerine, water, alcohol, etc., or even air; then through the cover-glass, and finally through air again. In every one of these changes light is lost. Owing to this loss of light, as well as for other reasons, it is not wise to use high power eye-pieces with dry objectives; besides which, as the eye-piece does not magnify the object, but only the image of it as produced by the objective, any errors of this latter are likewise magnified by the eye-piece.

To obviate in part this loss of light, what are called "im-

[1] It is possible, by means of thin india-rubber or paper rings slipped on to the shoulder of the objectives, to adjust their focal lengths with great accuracy. In case of moderate inaccuracy of *centring* the student is advised to study for himself the approximate position into which an object should be placed when using the low power in order to bring it into the approximate centre of the field when the higher power is turned into use.

mersion" objectives are now much in use. In these objectives the cover-glass and the front lens of the object-glass are connected by a drop of liquid. Such objectives are of two kinds: "water," in which that is the liquid used, and "homogeneous," in which the liquid is in general oil, or a mixture of oils. The homogeneous immersion objectives are dearer, less readily cleaned after use, and must be used only with the special fluid for which they are manufactured; but on the other hand they transmit more light, bear a stronger eye-piece, and, within certain wide limits, are independent in their working of the thickness of the cover-glass. Dry objectives of high power, and water immersions, are naturally dependent on the thickness of the cover-glass which the

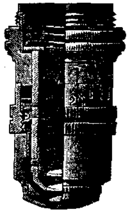

FIG. 1.—Objective with collar for correcting for thickness of cover-glass (Zeiss).

light-rays pass through after leaving the object. To provide for this, they are usually manufactured also with "correction collar," for use according to the thickness of the cover-glass, and at a somewhat increased price. The correction collar accommodates the objective to the thickness of the cover-glass which happens to be in use, by means of a milled-headed screw which turns it, and adjusts the lenses forming its optical system; and upon it are divisions and figures (see Fig. 1) which permit focussing for any given thickness (within certain limits) of cover-glass, where this thickness is known. A water immersion or a dry objective, without correcting screw, is made to suit a certain medium thickness of cover-glass, which is usually stated by the optician; and it is therefore preferable for the beginner, if he wants such

an objective, to use with it cover-glasses of this definite thickness.[1] But whoever does not fear the expense would do well to provide bimself at once with an objective for "homogeneous immersion".[2] They are all constructed without correcting screw, since, as already indicated, the thickness of the cover-glass, of course within the permissible limits, is of no importance. By selecting a single such objective, say $\frac{1}{12}$, and purchasing a series of eye-pieces, one can obtain a range of possible magnification such as could only be given by several water immersions, or dry objectives. A system for homogeneous immersion, provided it is perfectly constrncted, can therefore replace several systems of another kind.

As a set-off against this, the use of a homogeneous immersion, as indeed of a water immersion, requires special care, especially in cleansing both it and the object. It must be borne in mind that the immersion oil is a solvent of Canada balsam, so that if this is used as a mountant it must be protected by a layer of gold size. While an oil immersion is, as noted, largely independent of the thickness of the cover-glass, should this happen to be very thin indeed, it is well to compensate for it by slightly lengthening the draw-tube.

Even in the smallest stands mentioned above, objectives for homogeneous immersion can be used with great advantage without any special apparatus for increasing the illumination; but the highest capabilities of the homogeneous or indeed any system are only brought out by the use of a substage achromatic condenser. Several of the stands referred to above have substage condensers constructed specially for them, and at a very moderate cost.

The highest possibilities of a microscope are only attained by the aid of the comparatively new "apochromatic" objectives and their compensating eye-pieces, hut their high price, and the necessary accessories, may be taken for the present to place them outside the use of those for whom this book is intended.

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In c.
for him! On this subject see a note on page xxx.
when usiLeitz, of Wetzlar, produces a $\frac{1}{12}$ of remarkable excellence, which has the field whs test of manufacture for nearly thirty years. Price £5.

Owing to the now almost universal use of the standard screw of the Royal Microscopical Society of London, objectives of one maker can be attached to the instrument of another. A point of some importance to English purchasers of continental objectives is however this: the customary length of the tube of the microscope on the Continent is 150 to 170 millimetres (six to seven inches), and the objectives are constructed to suit this length. If the tube exceeds this length, it should be stated in ordering the objectives, that they may be modified to suit. This is especially needed in ordering objectives for homogeneous immersion. All the microscope stands mentioned above have tubes of continental length, and mostly of continental size.

Besides the compound microscope to which we have hitherto exclusively referred, a simple, or so-called preparation or dissecting, microscope is also more or less necessary. For all the purposes of this book, and, indeed, for most botanical purposes, whether in anatomy or morphology, a dissecting microscope of very simple construction is all that is needed, and two or three are illustrated on pp. 206-8. Most instruments for this purpose are unnecessarily complex and expensive. Some, for instance, are constructed to magnify up to 60, 80, or even 100 diameters; if such magnifying power is needed, the low power of the compound microscope will do equally well, dissecting being done upon the stage, but the arms being carefully supported. The student *can, indeed, entirely dispense with a dissecting microscope,* and dissect upon the stage of his larger instrument by the aid of his lowest power; but as the image of the object is inverted, and any movements he may make are likewise reversed, he would probably be at first somewhat perplexed. Practice will overcome this difficulty; or it can be cleared away at once by purchasing an "erector" for insertion in the draw-tube, costing usually 10s. or 10s. 6d. It is desirable likewise to have a low-power objective, *e.g.*, 2-inch or 1½-inch, though dissection with the 1-inch is perfectly simple. The lowest power eye-piece should be used. Dissection under the compound microscope has, with very small objects, this further advantage, that there is no chance of losing them in

removing from one instrument to the other. To this may be added perhaps another advantage, in that the working-table is not cumbered with an extra instrument. For dissecting with the microscope the wrists must be supported on a level with the object, or slightly below it. Some dissecting microscopes have arm-rests for this purpose ; blocks of wood of suitable height, or even stacks of books, will answer admirably.

A very necessary adjunct for microscopical work is a good magnifying lens, as it is often desirable first of all to study an object with it, afterwards using the microscope. The lenses of the dissecting microscope can be used as hand magnifiers, and low-power objectives likewise make good hand lenses. It is worth while, however, to get a lens magnifying about six diameters ; very convenient are the triplets, three lenses in a tortoise-shell case, usable separately or together, and sold at a price of about 3s. 6d. Remarkably beautiful are the Platyscopic Lenses of Browning (63 Strand, London, W.C.), magnifying fifteen, twenty, or thirty diameters, price 15s. each, and the Aplanatic Lenses of Zeiss, magnifying six, ten, or twenty diameters, price, 12s. or 15s. each. Becks', also, are now manufacturing some new hand lenses of very high quality at 11s. 6d. each.

As it is desirable that the student should from the first begin to draw the objects he examines (practical instruction in which will be found on p. 40 *et seq.*), it is desirable that he should have some form of drawing instrument to facilitate his work. Drawing can, it is true, be done without any such aid, but is more difficult. An apparatus for drawing (camera lucida) is constructed either for use with the body of the microscope placed horizontally, or placed vertically. Practically the latter is much to be preferred. Every microscope maker has appliances of his own make, but they vary very much in real utility. Two typical instruments, both manufactured by Zeiss, are figured on pp. 40 and 42, and their method of use is described.

A stage micrometer is likewise necessary to enable measurements of microscopic objects. This can be obtained from most opticians at a cost of from 5s. to 10s., and ruled up to $\frac{1}{1000}$ of

an inch. Zeiss has one at 10s. ruled up to $\frac{1}{100}$ of a millimetre, *i.e.*, about $\frac{1}{2500}$ of an inch.

Any steady table can be used by the microscopist for working, but it should be looked to that it is not too small, and not polished or varnished on its surface. This surface is best painted a dull dark colour. The table is so placed that if possible the microscope faces a window, and at not less than one yard distance from it. Any position of the window is good which allows a free outlook. From direct sunlight we protect ourselves by a white roller blind, which is best made of tracing-linen. The dazzling white light which we obtain when the direct sunlight plays upon the blind gives the most favourable conditions for observation with high powers. In using the microscope with light taken from a window, light *coming from any other direction should be avoided.* If a white roller blind is used to pass direct sunlight through, the eyes should be protected in some way from its glare. If artificial light is needed, microscope makers provide various forms of oil-lamp specially constructed for use. The light of these is very often improved by passing through a Florence flask filled with dilute ammoniacal cupric oxide, or through a disk of bright blue glass placed above the mirror. Artificial light, partly on account of the brightness of the field of view relatively to that of the room generally, and partly owing to the *strength of its shadows, is very tiring to the eyes,* and every care should be taken, in using it, to have the room generally as brightly illuminated as is the field of view.

The necessary object slides and cover-glasses can be obtained of most opticians. The former are procurable with either unground or ground edges at a cost varying from 2s. to 4s. per gross respectively. They are three inches long by one broad. The cover-glasses for ordinary observation should be about $\frac{3}{4}$-inch square; but the observer should also have larger ones for specially large objects or series, and also others somewhat smaller ($\frac{5}{8}$-inch square) which will usually suffice for permanent preparations. If we use powerful objectives, it will be best to obtain these cover-glasses of definite thickness. For the beginner, this is not of special importance; but the more advanced student will find it

advisable if possible to procure both object-slides and cover-glasses of a definite thickness.[1]

Cover-glasses, if thin, are not easy to manipulate without risk of breakage. On p. 10 will be fonnd described a method of cleaning them before use, which I [Ed.] have found useful for students. While keeping the stock in the thin boxes in which they are sent, a dozen or so are put out at a time into a kind of trough, made by taking a piece of hard wood, about 2½ inches long, 2 inches wide and 1 inch thick, and cutting out a portion of its top in the form of a slope or inclined plane, flush with the top of the block of wood at one end, and with a rim left along the sides and at the other end. Upon this flat sloping surface the cover-glasses lie quite safely, and can easily be moved with the finger, especially if slightly damped.

Further necessary are a plane- and some hollow-ground razors; a fine and a coarse pair of steel forceps and a pair with ivory points; a finely pointed pair of dissecting scissors, for which fine embroidery scissors will serve; a pair of needle-holders, somewhat after the fashion of crochet needle-holders, but so arranged that they will hold the finest needles firmly;[2] needles from No. 8 upwards, for these holders; some scalpels, some fine camel-hair or sable brushes;[3] a small vice, such as

[1] I had for years considerable difficulty in obtaining these latter in England (where, nevertheless, they are mostly made), but now obtain them direct from the manufacturers, Messrs. Chance Bros. & Co., Limited, Glass Works, near Birmingham. They are designated "No. 1," average $\frac{1}{160}$ inch (*i.e.*, 0·15 mm.) in thickness, and cost in square form 3s. 3d. per oz. They are made in various sizes. Chances also manufacture a somewhat thicker, and therefore stronger, cover-glass at 2s. 6d. per oz. Glass circles are somewhat dearer. Chances formerly manufactured specially thin microscope slides of 9 oz. crown-glass, ground and with polished edges, at 4s. 10d. per gross. These were of very fine quality and practically uniform thickness, but owing to lack of demand they have ceased to manufacture them.

[2] With care, needles can, by the aid of tweezers, be forced with their eye end into thin wooden paint-brush handles. According to the length that projects, the needle will be more or less stiff.

[3] For transferring sections from fluid to a slide the camel-hair brushes can be used as described on p. 27; or a simple "section lifter" can be made from a straight piece of stout copper wire 4 or 5 inches long, by beating out thin about half-an-inch or so of one end, cutting the edge smooth with scissors, and then bending the wire above the broadened part to an angle of about 125°.

used by watchmakers; some pipettes, glass tubes and glass rods; watch glasses of various sizes, and glass disks of suitable sizes for covering them;[1] china saucers as used for paint boxes; low glass bell-jars (receivers), in order to be able to fit up moist chambers; zinc frames, somewhat as represented in half-size in Fig. 2, on which to place the object-slides under the bell-jars;[2] two bell-jars of suitable height, under which to be able to place respectively the compound and the simple microscope; and lastly, elder-pith. For working, a tumbler of clean spring water is needed; a saucer is useful for dirty slides.

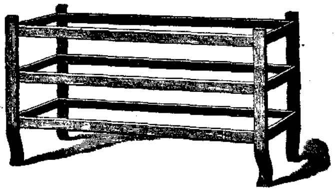


FIG 2.

The list of the necessary reagents is to be found at the end of this book. These reagents are in general best kept in small squat bottles, holding about an ounce, tightly corked, with the cork perforated, and holding in its bore a piece of thin glass rod with rounded ends, by which a drop of the fluid can be taken when required; or similar bottles, stoppered, with long tapering stoppers, can be obtained if preferred.

[1] Watch glasses can also be obtained, in pairs, ground to fit, and with brass-wire clip to hold them together. These are exceedingly useful for temporarily keeping a number of cut sections, etc., or for staining or other operations which require micro-sections or material to be exposed to the action of a small quantity of fluid for a considerable time.

[2] Slides can be also left upon these frames to dry, after permanent mounting. If the frames cannot be kept perfectly steady, the slides may wriggle off in time; to prevent this, sheets of paper ½ inch wider than the frame can be bent over them on each stage, and the slides laid across these. By using blotting paper for these sheets, and keeping wet, the bell-jar can be converted into a convenient moist chamber for a number of slide cultures at room temperature.

Where the word "alcohol" or "spirit" and not "absolute alcohol" is used, strong methylated spirits can always be understood, and is far cheaper. The alcohol on the working table is best kept in a "wash-bottle," made with a triangular Florence flask of about four ounce capacity, and fitted with a cork in preference to india-rubber. Another wash-bottle, which, for distinctness, should somewhat differ from this in shape, should contain distilled water.

In arranging the table for work, while the microscope is placed in front of the worker, and near the edge of the table, the space at its right-hand side should be reserved for books, drawing appliances, etc., while all the working materials which are liable to make even the slightest mess should be rigidly confined to the left-hand side.

For the preservation of permanent preparations, many kinds of cabinets and cases are advertised. It is very important to remember that the objects should be kept in a horizontal position, and should be capable of ready supervision. One of the best for this purpose at a moderate price is a cloth-covered box with cardboard trays, each covered by hinged cut flaps, which is made by R. & J. Beck, Ltd., price 8s. 9d., and holds 288; also made in smaller sizes; at 4s. 6d. to hold half a gross, and 5s. 6d. to hold 1 gross.

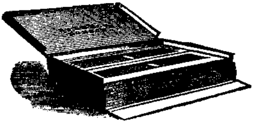


FIG 2.*—Beck's Cloth-covered Slide Cabinet.

CHAPTER I.

THE COMPOUND MICROSCOPE—STRUCTURE OF STARCH.

Principal Materials Used.

A fresh Potato.

Commercial potato-starch.

A bean; or bean flour.

West Indian Arrow-root (*Maranta*).

Grains of Wheat.

Grains of Oat.

Stem of the sun-spurge (*Euphorbia helioscopia*) and of *E. splendens* or *E. Jacquinæflora*. Fresh. (Other species can replace these if necessary.)

Principal Reagents Used.

Potassium-iodide iodine solution (hereafter called simply "iodine solution") —Alcohol iodine—5·0-10 per cent. potash—Diastase.[1]

Structure of the Compound Microscope.—We will first become acquainted with the separate parts of the compound microscope, and their use and purpose, taking as our type instrument Beck's "London" Microscope, No. 1125.

We can distinguish in it (Fig. 3) a solid horse-shoe **foot**, upon which is hinged or jointed the **pillar**, from which are supported the optical appliances of the instrument, and which, by means of the joint, can be placed in an inclined position, or thrown back to the horizontal. Projecting from the front of the pillar, just above the joint, is the **stage**, upon which the specimen we are examining is placed, and can be fixed in position there, if desired, by means of the two **clips** seen upon the upper side. When the microscope is used, as ordinarily, in a vertical position, these clips may well be removed. From the front part of the pillar projects a bearer,

[1] The following reagents or materials are constantly in requisition; hence it is assumed that the worker always has them at hand, and they are not mentioned in the chapter headings:—A wash bottle of methylated alcohol and another (or a tumbler) of water, absolute alcohol, glycerine, glycerine jelly, potassium-iodide iodine solution (commonly called "iodine" in the text).

which carries the **tube**, at the two ends of which are the lenses for magnifying the object. The lens, or system of lenses, screwed in at the lower end of this tube, is the **objective** or **object-glass**; the system of lenses inserted into the upper end is called the **ocular** or **eye-piece**. The light is directed upon the object, and is transmitted thence through objective, tube, and eye-piece, by means of a **mirror**, which is suspended by a short jointed arm from the

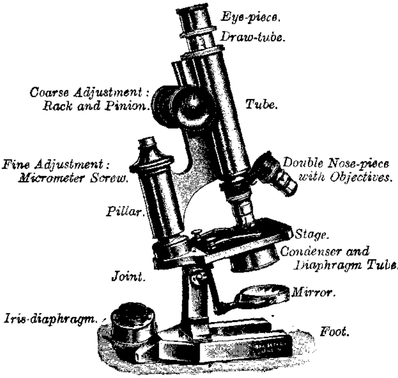


Fig. 3.—Beck's "London" Microscope, No. 1125, with $\frac{2}{3}$ and $\frac{1}{6}$ inch objectives in double nose-piece and iris-diaphragm. Half natural size. For full description see text; and price see p. xxi.

lower part of the pillar. This mirror can be turned in two planes, so as to reflect light coming from any direction; and the arm is jointed, so that the light can be thrown obliquely upon an object, though for ordinary student work this is rarely needed. Under the circular hole by which the light, on its way from the mirror, passes through the stage, is a tube or sleeve, in which can be inserted an **iris-diaphragm** (shown apart, near the foot), the plates of which can be opened, narrowed, or nearly (or quite) closed by a projecting lever handle shown in the figure; and thus the quantity of light thrown upon the object can be regulated at will.[1] For a small extra

[1] In the cheaper forms of microscopes of many makers, instead of the iris-diaphragm there is a fixed rotating wheel-diaphragm, with perforations of various sizes.

charge, the makers supply a **condenser**, with iris-diaphragm, to fit into this sub-stage tube, by means of which light can be concentrated upon the object; and the student is strongly recommended to purchase this additional appliance as soon as possible, since by its aid, and regulation by the iris-diaphragm, a very wide range of illumination becomes possible. Its value is very great in dull weather, or with high powers. The mirror-frame bears two mirrors, that on the one side being flat or *plane*, that on the other side *concave*. The former may be used for work requiring low magnification only, or when a condenser is in use; the latter for high-power work. There will be no difficulty in "finding the light"; *i.e.*, reflecting it properly through the microscope, when the eye-piece only is in position, without objectives, and it is not amiss to practise this.

The object, illuminated by light from the mirror, is magnified by the **objective**, and the image thus formed is again magnified by the **eye-piece**. With this instrument two objectives are supplied, a *low power* of $\frac{2}{3}$ inch. (16 mm.) and a *high power* of $\frac{1}{6}$ (4 mm.) focal distance. The makers allow some choice of objectives, but this is the best combination for ordinary purposes; others can be added later, if required. To obviate the necessity, with every desired change of objective, of unscrewing the one and replacing by the other, the microscope should have a **double nose-piece**, shown in position in the figure. This is screwed into the lower end of the tube, and the two objectives are screwed into its sleeves. With slight lateral pressure the nose-piece rotates in its centre, and one objective replaces the other, in position for use.[1] When well constructed this nose-piece is accurately centred, so that the optical axis of one objective exactly replaces that of the other, and with change of objective the object still remains in the field of view; and is also tight fitting, so as to exclude dust; but exact accuracy can hardly be expected in a low-priced instrument. If the microscope is in frequent use the objectives can be left in the nose-piece; but if the microscope is not to come into use for some time, it is better to unscrew them, and place them in the small brass boxes provided for the purpose. One eye-piece (No. II.) is provided with the instrument, but it is better to have Nos. I. and III. also

[1] The double nose-piece must be used with care, especially if too much mounting fluid be used, so that some flows out from under the edge of the cover-glass. With a thick fluid such as glycerine (so often used as a mountant) the extruded drop may be so high as to come in contact with the front lens of the high-power objective as it swings into or out of place, necessitating its removal for cleansing, and the attendant risks.

(5s. each). This would give a linear magnification ranging from 54 to 516 diameters; and a glance at the figures in this book will show how rarely the latter magnification is exceeded. Preferably, No. I. should be in general use.

The microscope is provided with two kinds of **focussing adjustment**, respectively known as "coarse" and "fine". The *coarse adjustment* is effected by **rack-and-pinion**,[1] worked by either of a pair of large milled heads; the *fine adjustment* by means of the **micrometer screw**, usually placed at the top of the pillar. Focussing, especially with the high power, should be performed very carefully, since it may be fraught with danger to preparation and objective. Hence the beginner will do well to give some preliminary attention to its method, though little difficulty will be found in dealing with the low power. The method of this preliminary training will be explained in connection with our first actual microscopical study.

It will be noticed that the tube of the microscope can be lengthened by a graduated **draw-tube**; the principal object of this is to allow a short compact tube to be so prolonged that the objective and eye-piece are at the distance apart which best suits their construction. In this particular microscope this distance, after allowing for the thickness of the double nose-piece, will be at the line marked 155 mm., or, if without nose-piece, 170 mm. It may be

[1] In cheaper instruments the *coarse* adjustment is effected by the tube sliding up and down in a sheath, which is often cloth-lined. The tube is best made to slide by a slight zig-zag movement, the left hand holding the microscope steady by being placed upon the stage or foot, and the right hand doing the focussing, the second finger-tip of this hand resting upon the tube where it enters the sheath, and upon the top of the latter as well, and serving as a friction brake to control the rate of movement.

The opinions of practical teachers on the desirability of a learner having, from the first, a rack-and-pinion instrument vary widely, though not so much as formerly. Laboratory instruments are still often unprovided with it, probably owing mainly to considerations of first cost. My own experience [ED.] of many years' use of both types of instrument is that *provided focussing up be practised* (as explained a few pages later on), the advantages are greatly in favour of the mechanical method of movement; and this is particularly the case where the rack and pinion is cut obliquely (or diagonally) instead of horizontally. If, however, the beginner "focusses down" whether with or without rack and pinion, he is certain to frequently overpass the proper focal distance, especially when using the high power; and, should the objective come into contact with the cover-glass of the micro-preparation, the results with rack and pinion are likely to be much more serious than with the sliding tube.

The experienced worker may focus up or down with almost equal safety, and in practice is pretty certain to often focus by the downward movement; but to him the rack and pinion is essential. It is largely with the object of restricting as fully as possible the need for focussing with the high power that I advise the use of the double nose-piece, and the correction of the lengths of the objective settings by paper collars (as explained on p. 11, footnote 2), so that they focus automatically for the same plane.

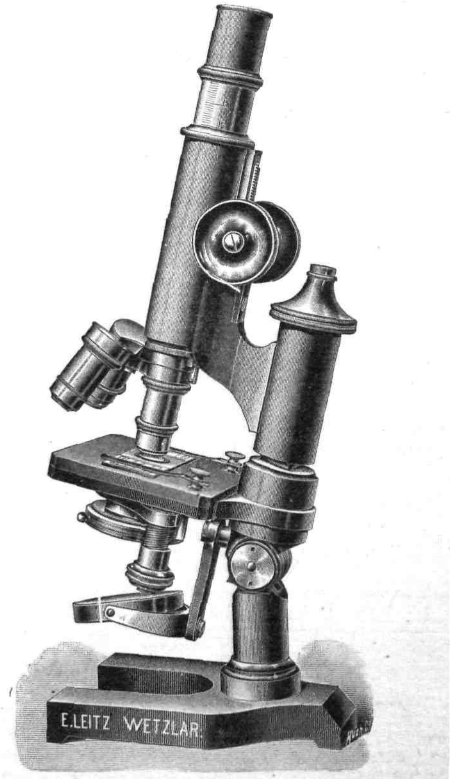
noted that using the draw-tube, *i.e.*, further separating eye-piece and objective, increases the magnification by the former of the image made by the latter; and the draw-tube is often improperly used as an aid to magnification. This habit should be avoided. It cannot be too much insisted on that the lenses work best at that distance apart for which they are constructed, and that any departure from this distance, in either direction, is disadvantageous. It must be remembered, too, that *increased magnification is not, per se, an advantage.* The constant tendency of the beginner is to magnify up to the utmost limits of his instrument. Clearness of vision is what should be aimed at; size may easily be purchased at the expense of this. No one would think it an advantage to use a magnifying lens with print which can quite easily be read without it!

One final word of warning. *A microscope should always be lifted by the foot, never by the body above the joint.* In this latter case, the strain of the weight upon the micrometer screw fine adjustment may gradually destroy its delicacy of movement.

Microscope for Higher Work.—The microscope described above is capable of doing satisfactory work in most departments of anatomical botany, and also of being improved by various subsequent additions; but for more serious work a better *stand*, with accessory apparatus, should be used.

A very excellent medium-priced microscope for higher student purposes is the Stand II.*a* of E. Leitz, of Wetzlar. The workmanship and optical appliances of this firm have a very, and deservedly, high reputation; and we will select this instrument as typical of those capable of use for all ordinary forms of higher work, and to which additional appliances can at any time be supplied. It is represented[1] in Fig. 4. This microscope (Fig. 4) has a coarse adjustment by rack and pinion, instead of by sliding-tube, and an exceptionally good fine adjustment by micrometer screw of delicate construction, situated at the top of the pillar. It has a draw-tube, with millimeter divisions engraved upon it. Under the stage is an illuminating apparatus on the Abbé principle, with condenser and iris-diaphragm fixed together, so that they can be withdrawn for oblique illumination, for which, also, the mirror is capable of lateral movements. This illuminating apparatus

[1] For price, etc., see Introduction. In common with foreign microscopes generally, the horse-shoe form of foot is retained, stability being obtained by weight rather than by form; and the jointing of a single vertical pillar as a means of inclining the body might with advantage be abandoned for some other method of suspension.



Stand II.*a* of E. Leitz, Wetzlar, with triple nose-piece, three objectives and sub-stage illuminator For description see text.

is moved on the optical axis of the instrument by means of a lateral screw showing in the figure, so that it can be focussed upon the object in accordance with the thickness of the micro-slide; and in place of it ordinary diaphragms can be readily used. A ring under the iris-diaphragm serves for the reception of a dull or coloured glass disk. The mirror is plane on one side (for low power work) concave on the other. When using the condenser the plane side of the mirror should be used, and the amount of light regulated by the iris-diaphragm. For low powers this should have its opening contracted, for high powers widened; if an oil immersion lens is used with stained preparations (particularly with bacteria), the iris-diaphragm can be left wide open.

The lenses of Leitz are, for their price, of exceptionally high quality. Any eye-pieces or objectives can be purchased, but for initial use perhaps the best combination is objectives 3 (= approximately $\frac{2}{3}$ inch of English classification) and 6 (= $\frac{1}{6}$ inch) or 7 (= $\frac{1}{8}$ inch approximately); and eye-pieces Nos. 0, II. and IV. Without the use of the draw-tube this then gives the following range of magnification: objective 3 and eye-piece 0 = 46 diameters; 3 and II., 70; 3 and IV., 105; 6 and 0, 210; 6 and II., 330; 6 and IV., 480; or 7 and 0, 270; 7 and II., 440; 7 and IV., 625. These objectives work best with the same tube length as in the instrument previously described, and the use of the draw-tube for further magnification is subject to the same criticisms. The double and triple nose-pieces of this firm are also of exceptionally good construction.[1] Various supplementary appliances, *e.g.*, for drawing, polarisation, etc., or for still higher magnification, can be added at any time, as finances allow.

To place the Microscope for Work.—The microscope can be used either by solar or artificial light, but where possible the former is to be preferred. The microscope is placed opposite to a window, and at a distance of about a yard away from it, and in such a position that the direct sun's rays do not fall upon it. If the direct sun's rays fall on the window an admirable screen, producing perfect illumination, is provided by a sheet of glazed linen between microscope and window; much the same kind of light, in fact, as is produced in the ideal illumination from a white cloud. If desired the microscope can be used left hand to the light; but front position is preferable. While looking down through the microscope, we adjust with the fingers the position and slope of

[1] In ordering at the same time nose-piece and objectives, the maker will adjust them to one another in respect to centre and focal distance.

the mirror till the field of view appears bright and equally lighted. If there are initial difficulties, remove the objective, or twist the nose-piece crosswise, and adjust the mirror with eye-piece only. If the mirror arm is jointed, see that you do not twist the centre of the mirror out of the line of the axis of the instrument.[1]

We will now commence our work. An **object-slide** or **micro-slide** is wiped clean with a soft cloth, and upon it, by means of a thin glass rod, a drop of pure or distilled water is placed.

Making a Preparation of Potato Starch.—Let us commence our study by the aid of a potato-tuber. We cut this through with a pocket knife, and transfer a little of the sap which exudes from the cut surface into the drop of water by means of the same knife. We then cover the drop with a **cover-glass.** This must also have been previously cleaned with special care, which is best done flat between the fingers by the aid of pieces of old fine linen or soft Japanese writing-paper. This operation is not so simple as it seems. If the cover-glasses are thin, they are very easily broken. A method I [Ed.] have proved for many years to be least destructive for beginners is to slightly damp the thumb and index finger of one hand, say the left, place over the hand a piece of linen or silk, and push it in between the thumb and finger so as to form a fold. Taking the cover-glass by its edges between the thumb and finger of the other hand, slip it horizontally into the fold of the fabric and bring the covered thumb and finger down upon it—one above and the other below—and rub them to and fro on its two surfaces. The fabric will cling to the slightly damp fingers, and the process becomes easy. Some use little pads between which the cover-glass is placed, and the pads then moved about over its surface. The cover-glass must be laid on the preparation as carefully as possible, so as to exclude air from underneath it. For laying on, it can be held between the index finger above, and a needle (in a holder) underneath. By pressing the thumb-nail against the end of the needle-holder, the latter can be gradually

[1] If *artificial illumination* is used, it will probably be most convenient to use the *edge* of a single wick paraffin lamp, and to pass the light through a blue screen, *e.g.*, such as a saturated solution of acetate of copper, placed between lamp and mirror, or a disk of blue glass (provided by the microscope maker) placed in a suitable ring between the mirror and the stage. In my own laboratory (Univ. Birm.) small Nernst lamps with ground glass spherical bulbs are in use, and do well. Some of the new forms of glow lamps, with large bulbs and specially prepared filaments (such as the Ediswan "Radiolite" lamp) give a beautiful white light, and I have used them with much satisfaction. In none of these cases should the filament itself be visible; and the part of the lamp whence light falls upon the eyes will, with advantage, be shaded.—[Ed.]

withdrawn when the cover touches the water, and this is gently lowered into its place. If the drop is of proper size no water will flow out from the side of the cover-glass. The size of the drop has usually to be estimated in respect to (1) the size of the cover-glass, and (2) the thickness of the preparation to be covered; but in this present case this latter is very small. If water does flow out it can be removed with blotting-paper; or it is better, indeed, to make a second preparation, as most of the grains which we wish to observe would be sucked out by the blotting-paper.

Using the Microscope.—We now place our preparation on the stage of the microscope, so that the object lies over the centre of the opening in the stage, and draw out the draw-tube to its proper distance. Examining our two objectives, it will be seen that one has a shorter body, or setting, and larger front lens, the other a longer setting and smaller front lens; the former is the low power, the latter the high power. As we shall see in their use, these two lenses are of different focal lengths (and working distances), and the length of the setting is so adjusted that when the two are screwed into a nose-piece, and slewed round so as to replace one another in use, the focussing of each should be approximately correct. Let us commence with the low power ($\frac{2}{3}$ inch), which is so constructed as to be focussed upon an object when its front lens is at a distance (working distance) from it of about $\frac{1}{4}$ inch, with which working distance it will be our first duty to become familiarised. Let the tube be lowered by the rack and pinion until the front lens of the objective is well within this distance, as judged by the eyes from about the level of the stage; then, while looking through the eye-piece, rack the tube up very slowly. Soon the position is reached in which the previously invisible object begins to show in the form of small grains. If, however, we find that we have withdrawn the objective to more than about $\frac{1}{3}$ of an inch from the object-slide, without having caught sight of the grains, these either do not lie in the field of view of the microscope,[1] or else we have raised the tube too quickly, so that the rapidly appearing and equally rapidly disappearing objects escape untrained observation. We will, therefore, try again, racking the tube down, as before, while carefully watching its position with the eyes near the level of the stage, till it is about $\frac{1}{8}$ inch from the object, and then begin anew to raise the tube, more slowly than before, while

[1] A great advantage in the use of potato sap for this preliminary study lies in the probability that the starch grains will be distributed under the entire surface of the cover-glass.

looking through the eye-piece. If you do not happen to see the object itself, the focus may often be approximately found by means of specks upon the cover-glass. If we do not succeed in this second attempt we may assume that the object does not lie in the field of view, which after all is a very limited one; and we must look for *it again after altering the position of the object-slide. After some* attempts the grains at length appear in the field of view, and we then discontinue racking the tube, that is, using what we call the coarse adjustment, and effect the fine focussing or adjustment by means of the micrometer screw (Fig. 3). This we turn in the first instance in either direction, but in case the object is made thereby more indistinct instead of clearer, we move it in the opposite direction. This adjustment, or focussing, is perfect when the figure appears as sharp as possible. In our example of a microscope stand (Fig. 3), the micrometer screw is at the upper end of the pillar, and this is its usual position.

Having now found, and carefully focussed upon, the object, make exact mental note of what interval there is between objective and preparation. As this *working distance* is a fixed one for the objective in question, the more exactly the student remembers it the less trouble there will be in subsequent operations. It would, indeed, be well to throw the instrument out of focus, and repeat the whole process over and over again, in all cases focussing by an upward movement of the tube. This method of *focussing up* is the only safe method for a beginner to adopt; this cannot be too fully insisted upon. It is well from the first to note what movement of the milled heads produces focussing up, or the reverse (focussing down), noting not merely the direction of movement of the milled heads, but the amount necessary to produce a given result, comparing in these respects the coarse and the fine adjustment, and repeating the movements until familiar with them. It is well also to practise *moving the micro-preparation about upon the stage*, by means of the fingers of either hand, or in the first instance perhaps of both, so as to realise what is done when its position is shifted. Remember that the magnification you are using will represent the number of times the actual movement of the fingers is multiplied in the apparent movement of the object; and you will notice at once that the direction of movement is reversed in the movements of the image. The student should get accustomed as soon as possible to this double phenomenon, multiplied rate of movement, and reversal of direction. Control of the hands for the small and slow impulses they give to the micro-preparation may be

best secured in this way: Lay the finger and thumb of each hand upon the stage, lightly resting upon the edges of the micro-slide *near the ends, but mainly resting upon the stage itself.* When moving the slide press the fingers and thumbs upon the stage; the friction will control the extent and rate of movement.

When the student is quite familiarised with the method of getting *a micro-preparation into focus with the low power, its working* distance from the objective, and how to shift the position of the preparation so as to place some grain or group of grains in a particular part of the field of view, the *high power* can be similarly *studied. We start with the low power in exact focus,* and then by means of the revolving nose-piece swing it out of position and replace by the high power. This latter should now be in approximate focus—at a distance, that is, of about $\frac{1}{50}$ inch[1]; while, as already pointed out, *if the nose-piece is accurately centred*, an object placed in the centre of the field of view while under observation with the low power should also be somewhere in the field of view with the high power.[2] If the microscope is not provided

[1] As this actual working distance of the high-power lens is calculated from the object itself to the setting of the front lens of the objective, the proportion of this distance which is occupied by the cover-glass, *i.e.*, the thickness of this latter, is naturally of importance. With the low power this does not come into practical consideration. See also the footnote to p. 4, and *Introduction, p. xxx., footnote 1.*

[2] In both these respects, length of setting of the objective and centring of the nose-piece, faults will probably be found; but if nose-piece and objectives are purchased at the same time, and the request is made, makers will sometimes match them properly. It is but natural that defects may be greater in the lower-priced instruments; but the student can himself to some extent make the needful corrections. This will be relatively easy for the private worker who, in the nature of things, will use the same instrument when working; and will be easy also for the laboratory student in those cases where a specific instrument is allotted, and not shared with others. (It would be well for this always to be the case, since it gives also a sense of responsibility for the safety and proper use of the instrument.) Every microscope has its individual peculiarities or defects in construction, greater or smaller. Defects *in the centring of the nose-piece* can be compensated for very easily by fixing attention upon some object, or group of objects, situated in the centre of the field of view when the high power is in use, and noting what position it occupies when the low power is swung on. This particular position in the field of view of the low power, then, represents the centre of the high power field; and any object placed in it, while the low power is in use, will be in the best position for study when the high power is swung on. For the same instrument this correction is a fixed one; and, since low-power study of any preparation always precedes the use of the high power, ability to make it gives great advantage to the use by a student of one and the same microscope during any definite period of his studies. *Defects in the length of the objective setting*, arising from one being somewhat shorter than it ought to be, may be corrected, in gross cases, by placing one or more rings of paper, or preferably of glazed silk or linen, upon the shoulder of the too-short objective, where it screws into the nose-piece. But for student purposes there are advantages in the high-power setting being slightly too short, since it more easily clears mounting fluids, etc., when being swung into use; and with a little very care-

with a *double nose-piece*, it will be necessary to raise the tube somewhat, unscrew the low power objective, screw the high power in its place, and focus anew by the original method; that is, lower *the tube till the front of the objective as nearly as possible touches* the cover-glass, and then focus up, turning the milled heads of the rack and pinion with extreme slowness, and looking through the tube with intent care. In either case perfect the focussing with the micrometer screw fine adjustment, the milled head of which will be found at the top of the pillar. It will be readily understood that in using rack and pinion with the high power, *focussing up* is of graver importance, even, than with the low power, since the working distance is so very small, that overshooting the mark, with possibly disastrous results to specimen or objective, can occur much more readily. With studious adoption of this method of focussing up the student will learn to depend more and more upon the coarse adjustment for all excepting the final stage of focussing, even with the $\frac{1}{6}$ inch objective, and thus to prolong the working life of the fine adjustment. It is well to remember that by wear this gradually loses truth and delicacy, so that the student should avoid misuse of it; and above all avoid getting into the bad habit, while observing, of mechanically sawing it backwards and forwards. In this same connection it is well to emphasise the advice that, in lifting a microscope it should never be grasped by the pillar or tube, since this throws the whole weight of the heavy foot upon the point of the micrometer screw.

We assume that the grains have been found and properly focussed with the high power; and before repeating the same kind of practice as with the low power (with proportionately greater caution), it may be noted that as the magnification of the object is now greater, the illumination of the figure appears feebler. It may be possible to increase the light by slight adjustment of the mirror; but in attempting this without due caution it will be found very easy to lose the light altogether; in which case we may find it needful to revert to the old plan of displacing the objective (*e.g.*, by setting the nose-piece crosswise) and finding the light through eye-piece and tube only. For both low and high powers, the amount of light transmitted through the microscope can be controlled by the diaphragm,

ful practice, when the same microscope is always in use, it can be determined exactly what proportion of a complete turn of the milled heads of the coarse adjustment (rack and pinion) is needed to lower the tube to a sufficient degree to bring the objective into as approximately exact a focus as very accurate construction of the objective-setting would itself give; and this method can then always be brought into use.

which for general purposes should be half-open, and the aperture reduced in case of need. A very little experimentation with the diaphragm will soon convince the student that the *control of light* is a very important aid to clear seeing. Light may be strong enough to render an object invisible; and for every case there is a certain strength of light at which the details of structure are best seen. This is of particular importance when using a sub-stage condenser. Light needlessly strong is also injurious to the eyes.

We now begin the actual study of the micro-preparation. The learner should accustom himself, so far as his eyes are equally good, to observe with the left eye. The right eye is thus kept free, and can be used in drawing, while he continues to observe with the left eye. Many of the drawing prisms and appliances for the microscope, moreover, are constructed for left-eye observation, and those who work with the right eye should intimate it in ordering such drawing prisms. The learner should also keep open the eye which is not in use. At first the surrounding objects, which are figured in the retina of the eye, will disturb him; but he will soon overcome the difficulty of concentrating all his attention on the eye engaged in observation, and temporarily suspending the activity of the other.

Potato Starch.—We readily recognise that the colourless bodies which occupy the field of view of the microscope are solid, and that they show lamination. They are **starch grains.** We slowly move the object-slide about in order to find a place where the grains are not too crowded, because in such a place it is more easy to fix attention upon a single grain. We select for careful study a grain which shows lamination with especial clearness. As the movement of the object-slide under the microscope appears to be reversed, we shall at first find some difficulty when we try to place a particular selected grain in the centre of the field of view so as to examine to greatest advantage; and we shall have as quickly as possible to accustom ourselves to control the slight movement upon which it depends. If we have found a specially favourable grain, we may magnify it still more by now removing the weak eye-piece and replacing it by a stronger, if we have one. If the eye-piece fits at all tightly, hold the tube of the microscope firmly while you do this, or the focussing may be altered, and the objective possibly forced down on the preparation. With good lenses the figure still remains good, although the brightness of it and of the field is diminished. We endeavour, so far as possible, to obviate this by improved adjustment of the position of the mirror. The illumination can be immensely improved by means of a sub-

stage condenser, should the microscope be constructed to carry one.

Sometimes after focussing the preparation, or after moving it, the figure may be seen to have lost its clearness. In all probability this is because some of the mounting fluid from the preparation has got upon the lower lens of the objective. This will very readily occur if too large a quantity of fluid has been used, and some has run out from under the edge of the cover-glass; or if pressure, *e.g.*, of the objective upon the cover-glass, has forced some out. Should this be the case, we must withdraw the tube from its sheath, or else remove the objective, or turn the nose-piece round; and, if our supposition is correct, wipe the front lens of the objective with a clean and often-washed piece of linen rag, or Japanese rice paper, or, better still, rub it with a freshly broken surface of a piece of elder pith.

The starch grains of the potato-tuber attain a comparatively large size, and show comparatively clear lamination. The laminæ are recognisable because, by reason of their varying density, they refract the light to differing degrees. They are excentrically constructed, as their organic centre, or hilum (*c* in *A*, Fig. 5) is not the geometrical centre, but lies considerably nearer to one end. The layers show with varying degrees of sharpness (*A*); between those more strongly can be seen others less strongly defined. Towards the surface of the grain the layering becomes indistinct. For optical reasons, and on account of its lesser density, the organic centre, or hilum, appears somewhat reddish. This is clearest when it is hollow, and it then shows either as a reddish dot, line, cross, or star with dark outlines. The layers immediately surrounding the nucleus are developed concentrically; soon, however, the excentricity begins to be produced by the layers thinning off towards one end of the grain, or possibly even in

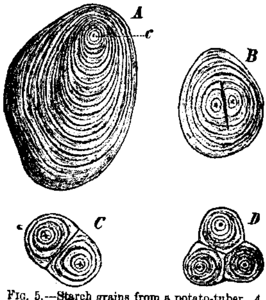


FIG. 5.—Starch grains from a potato-tuber, *A* simple grain, *B* half-compound grain, *C* and *D* entirely compound grains. *c* the nucleus (× 540).

some cases disappearing altogether. At this more feebly developed end of the grain, which we will call the anterior end, the layering is indistinct—the individual grains vary considerably in size, and deviate, moreover, from one another in outer form to a not inconsiderable extent, and show the layering with various degrees of sharpness. In the smallest grains the layering is hardly recognisable.

Between the starch grains in most preparations will be found rounded bodies, which, with a median focus, show a small, round, bright centre, and a broad, dark margin; this last is black at its inner edge, dark grey outwardly, and interrupted by clear intermediate rings. These structures are **air bubbles**, enclosed in the mounting fluid. Their appearance under the microscope is so characteristic that, once known, they can scarcely ever be confused with other appearances. The rays of light which pass out of the denser medium into the air bubble are, with the exception of the central ones, so strongly refracted (or bent) that they cannot pass into the objective, and hence the broad dark rim, and the comparatively small bright centre. If by turning the micrometer screw right-handedly (as in driving a screw into wood), the tube is lowered, *i.e.*, "focussed down," so that the under part of the air bubble comes into view, the sharpness and brightness of the middle disk increases; it diminishes at the same time in size, while the breadth of the surrounding dark ring increases. If the screw is moved in the opposite direction, or "focussed up," in order to focus upon the upper part of the air bubble, the middle disk enlarges, but loses somewhat in brightness; a grey ring of varying degree of brightness arises round it; the surrounding rim becomes at the same time narrower.—You may possibly see in the preparation a dark band completely crossing the field of view, which represents the rim of a very large patch of air, and shows, *mutatis mutandis*, the same changes with focussing up or down.

Drawing the Object.—If you have found a clearly laminated starch grain it should now be drawn. In microscopical observation the greatest possible stress is laid upon drawing. By its aid we first learn to observe acutely, and many special features of structure first become evident to the observer when he concentrates his attention upon an object for the purpose of making a representation of it. Drawing, therefore, protects from cursory superficial observation, enforcing thorough incisive study of the image, and

sharpens more than any other means our power of observation. The learner should first endeavour to represent the object by freehand drawing. He may already possess sufficient drawing ability for this, but if not can readily acquire by practice the needed facility. The object should not be drawn too small, even if the observer believes he sees it very small. A correct judgment on the size of an object in the field of view of the microscope is only attained after long practice, and it is better at first that the learner should draw the object too large, in order conveniently to include in his drawings all the details. No less important is it to provide the individual parts of the figure with suitable distinctive terms, and that the name of the plant, the purpose of the observation, and the most important conclusions, should be clearly noted by the side of the drawing.

The starch grains of the potato are only slightly flattened, as can be easily demonstrated if, while examining, you push carefully with a needle against the edge of the cover-glass, and so set the grains rotating.

Besides the simple grains (as at *A*, Fig. 5) will be found also, after some search, **semi-compound grains** (as in *B*), which enclose two, rarely more, organic centres or hilums. Each hilum is surrounded by a number of layers peculiar to itself, and both together by a smaller or larger number of common layers. Not infrequently the two inner systems of layers are separated by a cleft, extending as far as the outer, common, layers (*B*). The number of layers peculiar to the single grains, as well as of the common layers, varies according to circumstances.

The completely **compound grains,** which are found far more commonly than are the semi-compound, consist of two (*C*), less frequently of three (*D*), rarely of more than three component grains. In contradistinction to the semi-compound grains, the common layers are here wanting. The layers are most strongly developed in the line connecting the centres of the component grains; these latter, therefore, turn their posterior ends towards, and their anterior ends away from, one another. The line of separation between two component grains often broadens inwards into a cleft.

For comparison we now put up a preparation of potato starch which has been preserved in an air-dry state, transferring a trace of the powder into a drop of water. As the object-slides may

vary in thickness, it is advisable to raise the tube of the microscope before placing under it a new preparation, though this is not, of course, necessary when using a low power.

As the first preparation will be required again, we place it in a large **moist chamber.** This moist chamber consists in a deep plate (*e.g.*, soup plate) and a bell-glass (or *cloche*) with knob. On the plate stands a zinc frame, such as is described and figured in the Introduction (Fig. 1), and water is poured into the plate till the bell-shade has its lower edge quite immersed.[1] The preparation is laid upon the frame. Before this, however, we make sure that the drop of water under the cover-glass of the preparation is not already partially dry. If this should happen, we place a small drop of water on the slide at the edge of the cover-glass, so that it may be sucked in by capillarity. We also mark the slide, preferably with one of Faber's coloured pencils which write on glass, or upon a scrap of gum-paper attached to the slide, so that the preparation may not afterwards be confused with others.

Upon examination of the new preparation we shall find that the lamination of the starch which has been preserved air-dry is at least as clear as in the fresh grains. This preparation also we place in the moist chamber.

Bean Starch.—Now make a preparation of air-dry bean flour (*Phaseolus vulgaris*). The grains (Fig. 6) examined in water appear oval or circular; they are slightly flattened; a certain medium size predominates. The lamination is very clear and very uniform; the lamellæ show a well-nigh equal thickness. The hilum is central, and the structure concentric. The hilum of grains examined in water is hollowed, more isodiametric in the rounded, and elongated in the oval forms. From this hollow extend radial clefts, which cut through the layers at right angles, and, thinning off, reach almost to the periphery of the grain.

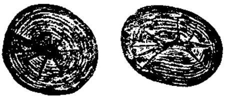


FIG. 6.—Starch grains from the cotyledons of *Phaseolus vulgaris* (× 540).

Now lay a trace of this bean flour in pure glycerine instead of

[1] This last precaution (immersion of the rim) is not so essential in the relatively moist air of an English room or laboratory. It is most convenient to use a soup-plate with regular rim, on which the bell-jar rests closely, and pour water into the bottom of the plate. If the preparation has to be kept for many hours, greater precautions against drying may be needed.—[ED.]

in water. In this mountant the starch grains appear on the average smaller; their lamination can scarcely be recognised; the inner hollow and the clefts are wanting. These were formed, therefore, as the result of the swelling of the grain under the influence of the water.

Arrowroot Starch.—West Indian arrowroot, also called in short arrowroot, prepared from the rhizome of various species of *Maranta*, especially of *M. arundinacea*, is easy to obtain in shops. Observed in water, the grains show great similarity to those of the potato; but they are usually less clearly and more uniformly laminate, somewhat more rounded, on the whole smaller, also more uniform in their size. At the position of the hilum is usually found a cleft, in the form of a wide-open V.

Wheat Starch.—Wheat meal shows the layering very badly; as, on the whole, most favourable, we select for observation the starch grains of *Triticum durum*. We halve the grain of wheat with the pocket knife, scrape off a little substance from the cut surface, and put it in the drop on the object-slide. The large starch grains are circular, discoidly flattened, and regularly laminate (Fig. 7, *A*); but the layers are usually difficult to see. In many grains they will, however, be recognised, as well as the central hilum. Occasional grains show also upon their surface a beautiful reticulation, more or less regular, and occupying only a small portion of the surface of the grain. This reticulation is formed by a network of small ridges, and, similarly, the meshes represent very shallow hollows. As a characteristic appearance, there will be found in the preparation, besides the large grains and almost without transition sizes, small grains with clear rosy hilum, but without recognisable lamination. Several such grains are represented at *B*. In many preparations compound grains are not rare; but usually they are sought for in vain, as they have fallen into their component grains.

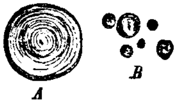


FIG. 7.—Wheat starch from *Triticum durum*. *A*, a large, *B*, small grains.

Oat Starch.—We shall best study the starch of the oat (*Avena sativa*) if we halve an oat grain, taking a little of the contents for observation under water. Compound grains are here met with in great beauty, as represented in the adjoining figure. The size of these compound grains varies, and similarly also the number of constituent grains entering into their composition. Fig. 8, *A*,

represents such a compound grain of medium size. The individual component grains appear polygonal, separated from one another by clear-looking boundary lines. Amongst the great grains are seen small ones, also compound, down to such as consist of but two component grains; lastly, also, quite simple ones, and besides these also numerous angular grains (*B*), which arise from the breaking down of the large compound grains in making the preparation. Amongst compound grains, a certain medium size, somewhere about our Fig. 8 *A*, is met with by far the most commonly. Lamination is not visible in this object; the hilum is only indicated in exceptional cases.

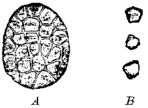


FIG. 8.—Oat starch from *Avena sativa*. *A*, a compound grain; *B*, its component grains (× 540).

Starch Grains of Euphorbia.—Of altogether peculiar appearance are the starch grains in the latex (milk) of the Euphorbiaceæ. A piece of the fresh stem of a spurge is cut off and the cut surface is immersed in a drop of water which we have ready upon the object-slide; the latex which flows out from the cut surface mingles with the drop. We can, for example, select for study a very widely-spread garden weed, the sun-spurge (*Euphorbia helioscopia*). In the latex, which appears distributed in the water as small drops, like an emulsion, we shall see isolated small rod-like bodies (Fig. 9). These are the starch grains in question. They appear pretty strongly refractive; lamination is indicated only in the most favourable cases; sometimes a longitudinal cleft is recognisahle in the interior of the grain. The size of the rods varies somewhat; many of them are slightly swollen in the middle.—Much more heautifully-formed grains of this kind are found in tropical Euphorbias. For examination we can choose *Euphorbia splendens*, or *E. Jacquinæflora* (preferably the former), both of which are commonly grown in plant houses for the sake of the brilliant scarlet bracts surrounding the inflorescence; and make the preparation in the same way as stated above. The starch grains which we now see (Fig. 10) are generally in the form of bones, with slightly enlarged ends; others may be shaped like rods, and still others have greatly enlarged ends, like dumb-bells, and in the swollen parts often permit something of

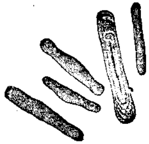


FIG. 9.—Starch grains from the latex of *Euphorbia helioscopia* (× 540).

the lamination to be recognised. Very frequently we see a colourless vesicle adhering to the side of the grain (*A*), the wall of which is referable, however, not to the substance of the starch grain, but to the plasma mass adhering to it.

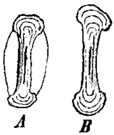


FIG. 10. — Starch grains from the latex of *Euphorbia splendens*. One of the grains has a vesicle attached laterally (× 540).

It must strike the observer that the small latex spherules distributed in the water are in tremulous motion. This is the so-called **Brown's molecular movement,** or "Brownian movement," which we can therefore take this opportunity of learning to recognise, and which is not a phenomenon due to life, but is referable perhaps to fine streams in the fluid carrying with them the minute globules.

Iodine Reactions of Starch.—Having learned this much about the form aud structure of starch grains, we will now produce some reactious upon them, and study the results under the microscope direct. First take a preparation of potato starch again out of the moist chamber. After we have focussed the microscope upon the grains, place a drop of a solution of iodine (in water, alcohol, or in watery iodide of potassium) at the edge of the cover-glass. For this purpose is specially recommended the use of a potassium-iodide iodine solution which is prepared by treating 0·5 gram potassium iodide and 1 gram iodine with a little water, then diluting with water to 100 c.cm., and leave it to stand on the iodine which separates out.[1] In using the reagent we must take special care that the drop does not run over the cover-glass, and thence, perhaps, upon the objective. If a drop runs upon the cover-glass, let it be immediately sucked off with blotting-paper. If the reagent reaches the objective, plunge the lower lens of this latter into pure water, or spray it with water from the wash bottle, and clean it afterwards with the piece of linen rag, or Japanese rice-paper, already recommended for cleaning purposes.

[1] I have prepared this form (Arthur Meyer's) of the well-known reagent by grinding 0·5 gram potassium iodide and 1 gram iodine together in a glass mortar, and dissolving in 1 c.c. water. If the potassium iodide solution is made weaker, as, *e.g.*, with 3 c.c. water, the iodine is not completely dissolved. The result, after addition of the 99 c.c. water, is to produce a weak potassium iodide solution which is quite saturated, possibly even supersaturated, with iodine, and in which metallic iodine is separated out in exceedingly fine particles.—[ED.]

In order to see the direct action of the iodine, await its penetration to some spot already selected and focussed upon—this spot, however, being chosen not too far from that part of the edge of the cover-glass at which the reagent is placed—and follow by movement of the object-slide the progress of its action. We see that immediately the influence of the iodine begins to make itself felt, the starch grains stain, first pale, then bright blue, and rapidly darken until they are black blue. At the first moment of the reaction the lamination shows up clearly—disappearing as the grains become opaque. With potassium-iodide iodine solution, in case this is added in considerable quantity, a dark-brown coloration of the grain is quickly induced. Similarly dry starch grains, which are exposed to the action of iodine vapour, become a very deep brown. If we add water to such a preparation, the brown changes rapidly to blue. If the action of the reagent does not proceed rapidly enough under the cover-glass, it can be easily accelerated by sucking it through by a fragment of blotting-paper placed at the opposite side of the cover.—The most beautiful violet-blue coloration of the starch grains is obtained, however, when a scale of iodine is laid amongst the starch grains in the drop of fluid under observation. The staining commences immediately in the vicinity of the scale.

The blue reaction of starch, therefore, occurs only in the presence of water. If air-dry potato or other starch be placed on a slide, covered with a cover-glass, and a little alcohol iodine run in, the grains are coloured a more or less deep yellow or reddish-brown. If, however, a little water be now run in, the blue colour at once appears.—A similar appearance is presented if potato starch in iodine, when heated for the purpose of swelling, is heated to dryness. The grains, previously blue, become brown, but the blue colour is restored on running in more water.—If iodised starch in small quantity is present in water, the blue colour may fade and disappear on heating, returning on cooling. This, again, is characteristic of the iodine reactions for starch.—We can stain with iodine the rod, etc., shaped grains of *Euphorbia* also, in order to demonstrate that, in spite of their unwonted form and scarcely noticeable lamination, these bodies are true starch grains.

Swelling (*Gelatinisation*) *of Starch.*—The phenomena of the swelling of starch grains under the influence of caustic potash solution (potassium hydrate) may now be studied. We will once

more take potato starch, place a drop of the reagent (5·0 or 10·0 per cent. solution) against the edge of the cover-glass, and await its entrance. The action must take place quite gradually, if it is to be instructive. We then notice, at the first moment of the action, that the lamination shows up more clearly; quickly, however, disappearing, while the grain increase in size. During this enlargement, which proceeds with more or less regularity, the hilum of the starch grain hollows out considerably, upon which the wall of the weaker side, that towards the anterior end of the grain, sinks into the hollow. Later on the regularity of the phenomenon disappears altogether, and the grain enlarges into a mass of considerable volume, and as clear as glass, the limits of which are ultimately scarcely recognisable.—In some respects it is an advantage in this reaction to use one of the preparations which has been stained pretty deeply with iodine. The potash destroys the colour, but it persists long enough to enable the swelling of the grains to be more easily followed.

Swelling by Heat.—Lastly we can endeavour by warming the preparation to cause the starch to swell, a method which is, indeed, in use in making paste. The preparation is warmed over a spirit or gas flame, without allowing it to boil, and taking care to replace by fresh water that which evaporates. If a temperature of about 70° C. (160 F.) is reached, the grains will be found to be swollen just as by the potash method. In this reaction, also, it is of advantage to treat the starch previously with iodine. According to the extent of heating the colour fades or disappears, though it may reappear on cooling.—In order to determine with accuracy the temperature at which starch swells, the preparation must be warmed upon a heating microscope stage.

•*Polarisation.*—If a polariscope should happen to be at our disposal it will enable us to get an instructive insight into the character and physical structure of starch grains. A polariscope consists of two parts, known as polariser and analyser respectively, each of which consists essentially in a prism of Iceland spar ("Nicol's prism"); the former is placed below the stage so that the light passes through it before entering the object. The latter is placed above the object, being either interposed between objective and eye-piece, or passed over the latter like a cap. If a preparation of potato starch be placed on the stage in polarised light, *i.e.*, with the prisms of the analyser and polariser crossed by partially rotating one of them, the field of view is darkened,

and the grains show a characteristic more or less symmetrical (according to the symmetry of the grain) dark Maltese cross upon a bright ground, the intersection of the arms of the cross being at the organic centre, the hilum of the grain. If the analyser be rotated the cross will also simultaneously rotate. The grains are therefore doubly refractive, which is taken to indicate a crystalline character,—that the grains are of the nature of sphaero-crystals, each lamina being composed of needle-shaped crystals radially arranged.

General Observations on Starch.—In our subsequent plant studies we shall come across starch grains in many forms; but it will be well to realise that these grains of comparatively large size and more or less distinct lamination are associated with places where starch is accumulated as a reserve store of food. In growing structures generally, in leaves and so forth, starch grains are small, but always to be readily recognised by the iodine reaction, though in some cases it may need to be aided by some process which brings about slight swelling, *e.g.*, very dilute potash, slight warming, or other processes to be learned hereafter. Or if the section in which the starch is be thin, a concentrated solution of iodine in potassium iodide will stain the smallest grains a very deep, practically a black, colour, so that they stand out distinctly amidst the brown protein substances.

In the processes of germination starch undergoes changes, being gradually converted into glucose or maltose, and then dissolved. In the living plant this probably takes place from without inwards, the solution either being regular, or local corrosion goes on so that surface hollows, or pits, or even fine canal-like openings extending even to the centre of the grain, are formed. Such corroded grains may be found occasionally in germinating seeds or potatoes, or may be produced by the action upon starch of a solution containing a special body, called "diastase". This solution may be prepared by an aqueous solution of commercial diastase with about ·05 per cent. of citric acid added, or by dissolving a little malt extract in water; for diastase is contained in, and can be extracted in large quantity from, malt or germinating barley. Starch grains which are thus being changed in the processes of germination lose in part or wholly the power of taking a blue coloration with iodine; they may show a partial or more complete red coloration, or even a nearly pure yellow colour. In this latter case we have what is called

a "starch skeleton," which may show the size and form of the original grain, and even stratifications, but in which its chemical constitution has been very greatly altered.

With this we close our first Lesson. Before we put the microscope on one side we carefully clean, in the manner before described, the objectives and eye-glasses, together with any other pieces of apparatus that we have used. Instead of again replacing the microscope in its cabinet, we prefer to place it under a glass bell-jar, which latter, in order to protect the instrument as much as possible from dust, can have its lower edge standing on felt or surrounded with a ring of chenille, such as is commonly used with glass shades for covering ornaments. Remember to lift the microscope by its foot, not by pillar or tube.

NOTE ON THE CARE OF THE MICROSCOPE (BY MR. ERNST LEITZ).

Dust is a disturbing element which makes itself soon noticeable. By turning the eye-pieces round or moving the specimen the seat of the obstruction is easily ascertained.

The condition of the objectives may be examined by holding them at some distance from the eye against a window. The small image of the window seen in the lens should be perfectly clear. A hand-lens of low power is particularly useful for examining lens surfaces.

Dust is removed from the lens surfaces by means of a fine dry camel-hair brush and by blowing lightly while brushing. If this will not remove the dust the lens surfaces may be breathed upon and gently wiped with a clean soft piece of linen. Particles of dirt adhering very firmly should be removed with linen damped with alcohol or xylol.

When examining objects treated with chemical reagents great care must be taken that they do not come in contact with the lens surface. Should this nevertheless happen the lens should at once be rinsed in water and carefully wiped. The use of very large cover-glasses is the best means of preventing such occurrences.

No fruitless attempts should be made to obtain lower powers by unscrewing the mounts of the objectives.

Before and after using the microscope it is wise to inspect the lenses and eye-piece and to clean them forthwith if necessary.

The stand too requires some attention. Its rack and pinion, micrometer screw and other important parts should occasionally be carefully cleaned and sparingly lubricated with oil which is perfectly free from acid, such as pure bone-oil (best jeweller's oil), now and then cleansing the oiled parts with benzine.

The stand is best cleaned with a soft piece of chamois leather. Canada balsam, immersion oil, etc., is removed with Japanese paper with xylol, but on no account should alcohol be used for cleaning lacquered parts. It is also well when wiping the stand to follow the grain of the lacquer.

Separate cloths should be kept for the objectives, eye-pieces and the stand, in a dust-proof receptacle. It is a bad habit to use ordinary pocket-handkerchiefs.

With proper care and attention to these hints there is no reason why a microscope should not preserve its fine optical qualities and elegant appearance

CHAPTER II.

RESERVE STUFFS *IN SITU*—ALEURONE GRAINS—PROTEIN CRYSTALS—FAT OILS—MOUNTING OF PERMANENT PREPARATIONS.

PRINCIPAL MATERIALS USED.

Ripe Peas.
Grains of Wheat.
Seeds of *Lupinus* (Lupin).
Seeds of *Ricinus communis* (Castor oil).
Sections of the same hardened for twenty-four hours in alcohol.

PRINCIPAL REAGENTS, ETC., USED.

Glycerine iodine — Alcoholic borax-carmine — Millon's reagent — Iodine-green, or Methyl-green—Magenta—Olive oil—Glacial acetic acid—Eosin—Logwood—1 per cent. osmic acid—Tannin—Ferrous sulphate solution—Xylol—Canada balsam.

The Pea. Section-Cutting.—We first of all study the Pea (*Pisum sativum*). A ripe seed is halved by a sharp pocket-knife, and in such a manner that the two fleshy cotyledons are cut across; smooth the surface and then take from it a thin cross section with a sharp, hollow-ground razor. On the subject of section-cutting with the razor the following points can be noted: 1. The cut surface should be moistened before cutting the section, usually with water, but in this case with glycerine, since the preparation suffers in water, and we shall observe it in glycerine. 2. The first section is not to be used, as the tissue here would be too much injured by the pocket-knife. 3. In such resistant tissues as that of the pea only very small and exceedingly thin sections ought to be taken, as the edge of the razor would be very easily notched. If the razor has gone too deeply into the tissue, and it is seen that the resistance to its progress increases, it is better to withdraw the razor, rather than force it to the end of its cut. 4. Unless the investigation requires it, it is advisable not to commence the section with the outer surface of the object

but rather to lay the razor on the cut surface, as thus a far firmer support is obtained for getting a thin section. 5. In order to get a really good section, that is one in which the individual elements of the tissue are not torn, the razor must not merely be pressed with its edge against the object, but at the same time drawn across it. It is well, therefore, in order to cut as freely as possible, to accustom yourself not to rest the thumb of the cutting hand upon the other hand. Instead of this, both hands can with advantage be rested against the breast, because lateral movement of the cutting hand is not hindered thereby. The side of the razor-blade should be laid on the index-finger of the hand supporting the object. 6. As it is difficult to hold so small an object as a half pea, especially when it is also so hard, sufficiently firmly between the fingers, it is recommended to use for the purpose the small hand-vice described in the Introduction. The half pea is therefore fixed sufficiently deeply in this. 7. It is not advisable to be satisfied with a single section, but to take a considerable number, in order to make choice of the best. The razor must be stropped from time to time upon a suitable strop. Those with a firm backing are preferable to the loose strap-shaped ones. The mode of sharpening razors upon a stone would be best learned from an instrument maker. It is, however, of great importance to bear in mind that the "hollow-ground" razors so much used should taper off uniformly to the edge, and that the grinding of a supplementary facet upon this edge should never be permitted. Barber-sharpened razors are very liable to this.[1]

[1] A few more practical hints on the subject of section-cutting by hand may be of use to beginners. The object to be cut should be held pretty firmly between the thumb and index-finger of the left hand, the index-finger being held as nearly as possible horizontally, and slightly bent, the thumb likewise very slightly bent, and with the joint depressed below the level of the finger, in order to ensure its safety should the razor slip. In holding the object to be cut, the side of the tip of the index-finger should be rather higher than that of the tip of the thumb; the razor being then grasped firmly but not stiffly, the blade held quite flat and horizontal, the edge towards the body; the index-finger of the left hand will serve as a table, on which the blade will lie, and thus be greatly steadied. The section should be cut by a single forward and lateral movement of the blade. With all objects which will bear it, the razor-blade may float with alcohol on its upper side, and the object should be similarly wetted; otherwise the object, as here, may be kept moist with water, or hard objects with glycerine. For this purpose two "wash-bottles" are a saving of time—one for distilled water, the other for alcohol.—[Ed.]

The section selected should be observed in glycerine, either concentrated or diluted with about one-third distilled water. Pure water should not be used, as it quickly sets up phenomena of disorganisation in the ground substance of the cells. The transfer of the section from the razor to the glass-slide is best made with a fine camel-hair brush. The section is removed by pressing the brush upon it and sliding it off from the blade. If it adheres to a sufficiently broad surface of the brush, "curling" of the section will be prevented; curling occurs very easily, on the other hand, if the section is taken directly by its edge with the tweezers and so transferred. The section adhering to the brush is immersed flat in the drop on the glass-slide, and the brush withdrawn sideways with a simultaneous twisting movement. If it is desired to turn a section over when on the object-slide, press the brush down on the object-slide so that it is in contact with the edge of the section, and then begin to turn it over away from the section. In this way the section will be very easily drawn upon the upper surface of the brush, and can then be turned over with it. Other similar "dodges" will soon be acquired in practice. After every time of use the brush must be most carefully washed in water. Very small or stiff sections can be readily lifted on a needle. Flattened needles, slightly bent, can also be used with advantage.

The Pea. The Cell and its Enclosures.—Examine the section of pea first with a low power to get a general idea of its nature, and to select the best place for study; then change to a stronger power. It proves to be a **tissue** composed of rounded **cells**. At the places where three such cells adjoin one another a triangular **intercellular space** (*i*) filled with air is present. The air appears black, like the edge of the air bubbles previously described; here it naturally must show the form of the space, since it fills it. The

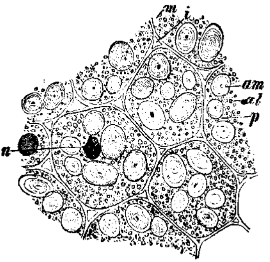


FIG. 11.—From the cotyledons of the Pea. *m*, cell wall; *i*, intercellular space; *am*, starch; *al*, aleurone grains; *p*, ground substance; *n*, nucleus. This last is shown by acetic methyl green (× 240).

wall of the cells (*m*) is pretty thick. In the adjoining figure the three middle cells are completely, the surrounding ones only partially, represented. In each cell can be seen the large starch grains (*am*), and with some care also the small grains (*al*) which lie between them. These small grains are, on their part, embedded in a very finely granular **ground substance** (*p*). From thin parts of the section many starch grains will have fallen out; a hollow of similar form and size in the granular mass will indicate these places. The small grains are **aleurone** or **protein grains**; they lie in the ground substance of the cell. If we run iodine solution, best in the form of glycerine iodine, into the preparation, the coloration which ensues gives us immediate information as to the individual constituents of the cells. The drop of iodine solution is placed at the edge of the cover-glass; as, however, the iodine solution diffuses very slowly in the glycerine, and it is not our present purpose to study the progress of the reaction, we accelerate it a little by slightly raising the edge of the cover-glass with a needle, and so facilitate the mixture of the iodine with the glycerine. A second needle placed at the same time against the opposite edge of the cover-glass prevents it from slipping. The starch grains colour blue to violet; the aleurone grains and the ground substance yellow. If sections of pea are laid in a drop of alcoholic borax-carmine solution, in a very short time the ground substance, and also almost simultaneously the aleurone grains, are stained dark red; the starch grains remain colourless. The reaction becomes especially striking if, after the section is thoroughly soaked in the carmine solution, this is replaced by dilute glycerine or by water. This is done by sucking out the carmine solution by a piece of blotting-paper placed at one edge of the cover-glass, while at the same time the water or dilute glycerine is run in under the opposite edge. If a section is placed in Millon's reagent, the starch grains swell very strongly, and become unrecognisable; aleurone and ground substance are immediately disorganised; the disorganised mass, however, after some time, takes on a characteristic brick-red colour. If still another section is laid in watery iodine green or methyl green, after a short time there appears in each cell, between the other constituents, a greenish-blue spot of rather indefinite outline. This spot is the **Nucleus** (*n*). The other constituents of the cell have not stained; the starch grains are just a little swollen (they show radial clefts, which are wanting in glycerine), and the aleu-

rone grains also have increased in size, and appear as if porous or even hollow. We recognise, therefore, in iodine green and methyl green reagents which in the present case constitute specific stains for nuclear substance. Simultaneously, it is true, the cell-walls also colour somewhat, but this does not diminish the value of these dyes as reagents for nuclear staining. The cell-walls appear of a beautiful bright-blue colour, and, as the result of this, are traced out in the glycerine preparations much more readily than before. The intercellular spaces also stand out more sharply. The figure becomes still more beautiful if to watery iodine green solution we add a little fuchsin (magenta) until the previously blue colour becomes distinctly violet, and then treat the section with the mixture. As before the nucleus and cell-wall stain a beautiful blue, but the aleurone grains and ground substance a deep red. If a small drop of this double stain be mixed on the slide with a large drop of glycerine, and the section then laid in it, the staining proceeds as above but much more feebly; the aleurone grains, however, by way of compensation, are not injured in the reaction.

In the brown reaction with iodine, the accumulation of stains, and the brick-red colour from Millon's reagent, we have become acquainted with the most important means whereby to recognise under the microscope **albuminous bodies**; for to these belong aleurone grains as well as cytoplasm and nucleus. The substance of the nucleus shows a specially strong affinity for certain stains. The constituents of protoplasm (cytoplasm, nucleus, chromatophores), are, however, in general only feebly stained when in the living state, and that only by certain stains; a notable accumulation of colour only occurs after death, though in many cases death may be brought about by the influence of the stain itself or of the medium in which it is dissolved. Aleurone grains, which are composed of inactive protein matter, take the stain with proportional rapidity. These aleurone grains consist chiefly of **vitellin.**

Endosperm of Wheat.—A grain of wheat (*Triticum vulgare*) can be recommended as a second object of study. The grain is first halved (across) with a pocket-knife, then the half fixed in a small hand-vice in order to have sections taken from it. This time it is desirable so to take the sections that a piece of the skin also is present on them. Before cutting, moisten the surface with glycerine, and observe the section in the same fluid (Fig. 12).

The "skin" is formed of dead cells pressed closely together (p), and represents the combined pericarp of the fruit and spermoderm of the seed; under it lies a layer of rectangular cells, which are densely filled with small aleurone grains (al). The aleurone grains are embedded in a finely granular ground substance. Then follow elongated, less regular cells, which contain large and small starch grains.

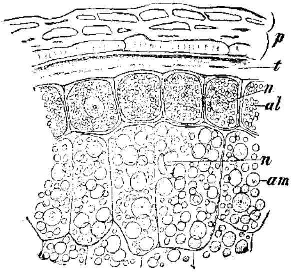


FIG. 12.—Cross section through a grain of wheat (*Triticum vulgare*). *p*, pericarp of fruit; *t*, testa of seed. In the endosperm cells succeeding to these: *al*, aleurone grains; *am*, starch grains; *n*, nucleus (× 240).

Mounting in Glycerine-jelly.—We will now "mount" a successful section of the wheat grain, so as to learn how to put up a permanent preparation, or, to use the common phrase, how to permanently "mount" a preparation. We will employ at first the simplest method of preparation, which gives a very satisfactory result, and enclose the section in *glycerine-jelly*. Place upon the object-slide so much of this jelly-like substance as we believe will suffice to form a drop. Then warm the slide slowly over the flame of a spirit-lamp, till the jelly has become fluid. It is very easy to take too much glycerine-jelly; if so a little can be removed, when fluid, with blotting-paper. In order to be able to choose a suitable quantity of jelly it is good to melt a quantity, and pour it out to harden in a thin layer on a plate; then with a sharp knife cut it into tiny cubes of various sizes. These can be kept in a closed bottle ready for use. If the jelly is taken each time from the original bottle, a needle will serve to cut out a suitable scrap. When, in time, the surface of the jelly gets much broken, the whole may be remelted by plunging the bottle into hot water, so as to remove the entangled air. Under any circumstances the selection of a piece of suitable size requires judgment, the necessary size varying with the size of the cover-glass to be used and the thickness of the section to be enclosed. —The section is then laid in the melted drop, and a cover-glass

placed over it. It is advisable first to warm the cover-glass a little, as otherwise air-bubbles will easily remain in the preparation, and for similar reasons it is desirable not to place the cover-glass on quite flatly, but with a slight lateral movement. If, in spite of this, air-bubbles are enclosed, the glass-slide can be warmed a little, and by careful raising of the cover-glass endeavour to bring the air-bubbles to one side. If the air-bubbles are not so placed as to interfere with the sections, removing them is not essential. If several sections are placed in the same drop they should be dispersed in it. No doubt it will often happen that, in laying the cover-glass upon them, the sections come into contact with or even overlie one another, and then if the cover-glass is raised on one side to secure order, a contrary result is often produced. Another comparatively simple method is therefore employed. By warming the glass-slide, make the drop as fluid as possible, and then, without lifting the cover-glass, pass in a hair from one side, and with this hair seek out the section to be adjusted, an operation which is usually successful. Before covering with the cover-glass it is, above all, necessary to make sure with the aid of a lens that no particles whatever of dust have found access to the drop of glycerine-jelly; any such should be removed with a needle. If in the process of these various manipulations the jelly has become too viscid, it can be again liquified by warming.

Preparations in glycerine-jelly do not require any "sealing," and provide, therefore, the simplest form of permanent preparation; and as most vegetable objects, even when stained, keep very well in it, while their structural characters even become often clearer, we can recommend this method of embedding in preference to all others. One or two precautions it is worth while, however, to bear in mind. Very watery preparations are best soaked in glycerine before placing in glycerine-jelly, or they may not preserve properly; or, if they are liable to collapse with this treatment, they can be first placed in very dilute glycerine, such as one part to nine of water, protected from dust, and the glycerine allowed slowly to concentrate, when the preparation can be transferred to the jelly. Further, as the jelly shrinks somewhat, after closing, and does this very slowly, it is well after the lapse of some months to surround the edges of the cover-glass with a narrow band of gold size, or other sealing material.

The preparation must now be labelled, preferably at both ends of the glass-slide. This may be done with gummed squares of paper, upon which must be written at least the name of the plant, the nature of the object, the direction of the section, if it be one, the medium in which it is preserved, any staining material used, and the date. A preferable method, however, is to use thin cardboard labels, made, for example, from Goodall's thin Bristol boards, and in size about the breadth of the glass-slide by about $\frac{5}{8}$ or $\frac{3}{4}$ inch. These can be cemented on to the slide by means of any one of the numerous forms of glass-cement, such as coaguline, mend-all, etc. A quantity of slides can be prepared at one time. The card-labels are cemented on both ends of the slide, and these are tied into bundles to dry under pressure. This method is very economical for keeping preparations, as all those of a similar kind can be tied into a bundle, with a plain slide over the uppermost, through which its label will be visible. Preparations preserved in glycerine do not then necessarily need to be closed with Canada balsam, provided the preparation is thin, so that little glycerine is used. If it is necessary to fasten card-labels with gum, it is best to cover each end of the slide first with a strip of gummed paper, the ends of which should overlap under the slide, and fasten the card-label on these; otherwise the label would readily spring away from the slide. The card can be fastened direct upon glass, without danger of "springing," if a solution be used composed of 100 grams of gum-arabic in 250 c.c. of water; to which is added a solution of 2 grams crystallised aluminium sulphate in 20 c.c. water.

If it be desired to approximately mark the position of a very minute object in a preparation, or of a special one amongst several sections upon the same slide, this may be done by drawing a circle (with Indian ink or a Faber's special pencil) upon the cover-glass, surrounding the position of the object.

Aleurone Grains of Lupinus.—Let us now take the seed of the white Lupine (*Lupinus albus*), or other allied species. Once more halve the seed across, and take sections from the moistened cut surface. Sections observed in water show in the cells rounded aleurone grains with vacuoles. In order to see the grains in their natural form they must be observed in glycerine. The grains then appear at first refractive, angular, gradually showing in their interior a fine network, granular. Lying closely together they fill up the cell; a small quantity of ground sub-

stance lies between them, more ground substance can be observed against the walls of the cells. The walls of the cells are very strongly thickened and pitted, a structure which we shall, however, study later upon a more favourable object. In iodine-glycerine the grains take a beautiful golden-yellow colour; in alcoholic borax-carmine they soon become red.

Aleurone Grains and Crystalloids of Ricinus.—In the next place we will take the seed of the castor-oil plant (*Ricinus communis*), from which we first remove the shell-like testa, then cut the kernel across, and make preparations from it just as above. The tissue of the endosperm is capital material to cut; it contains very much fat oil, and need not be moistened.—We will first examine *sections* in olive oil. The aleurone grains distributed in the cells of the endosperm appear in the form of small ovate bodies (Fig. 13). The narrower end of the grain is marked by a small rounded body, which appears as if hollow, because it is less highly refractive than the olive oil. This body is called the **globoid**; *it is mineral*, and it consists essentially of a double phosphate of lime and magnesia. The other constituents of the grain appear colourless, pretty highly refractive, occasionally more or less angular.—

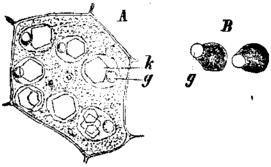


FIG. 13.—From the endosperm of *Ricinus communis*. *A*, a cell of the endosperm with its contents, viewed in water; *B*, single aleurone grains seen in olive oil; *g*, the globoid; *k*, the protein crystal (× 540).

We examine other sections in water. The globoids of the aleurone grains are now more highly refractive, and we can definitely see that they are solid bodies (Fig. 13). Each aleurone grain encloses one, rarely more, **protein crystal**, or **crystalloid**. The aleurone grains are embedded in a ground substance which is rich in **oil**. The water in which the sections are lying soon begins to displace the oil from the ground substance; this latter is gradually disorganised; great masses of oil collect upon and around the section. These in part cling to the object and to the glass, and have an irregular form, in part lie free, and then are spherical. Most of them appear turbid with numerous vacuoles. If the microscope be adjusted to an optical section of such an oil-drop, it appears bright grey, and is bounded by a narrow black

rim. If the tube be lowered, the black rim disappears, and the disk shows a somewhat brighter contour. If the tube be raised, the black rim becomes, on the other hand, broader. The oil-drops show, therefore, exactly opposite appearances to those which we saw on p. 15 were exhibited by air-bubbles. Air is less highly, oil more highly, refractive than water; hence their opposite relations; and these relations should be carefully noted for future use.

If we run absolute alcohol under the cover-glass of the preparation of *Ricinus* which we have placed in water, the preparation will "clear" somewhat; and, simultaneously, the protein crystals in the aleurone grains come out very sharply. They are now so clearly defined that this method of manipulation is recommended in order to study their form—tetrahedral hemihedra of the regular system. With longer action of the alcohol, the oil-drops disappear more and more, as castor oil, in contradistinction to other fat oils, is miscible with absolute alcohol.

Now make another preparation of *Ricinus* seed, lay it on the glass-slide in a drop of glacial acetic acid, and cover it with a cover-glass. The protein crystals swell quickly and disappear in the aleurone grains; these latter increase considerably in volume, the globoids show up very clearly in each aleurone grain. Oil drops are, however, not visible, because castor oil, again an exception to the rule, mixes with glacial acetic acid. Apart from this case absolute alcohol and glacial acetic acid, because normally they either not at all or but slightly dissolve **fat oils**, while on the other hand they are solvents of **ethereal oils**, are the best reagents for the purpose of distinguishing between these two classes of oil under the microscope. Of ethereal oils, the terpenes dissolve somewhat less easily than the others in both the above reagents. Chloroform and ether dissolve fat and ethereal oils equally.—To a preparation mounted in water run in alcannin tincture (alkanet root) diluted with water. The oil masses soon accumulate the colour and stain reddish-brown, a reaction which ethereal oils and also resins alike show.

Reactions of Proteids.—Aleurone grains of *Ricinus* and other plants form excellent material for the study of the general reactions of protein substances. Aleurone grains do not always contain enclosures, as a comparison of *Lupinus* and *Ricinus* (above) will show; we can therefore distinguish the general body

substance of the grain from the protein enclosures. The body substance of a protein grain consists of proteids of various degrees of solubility. In many seeds, as in the Pæony, the grains are wholly soluble in water · sections of such seeds would need to be examined in glycerine, or in alcohol, or other non-watery reagent; and if water be then drawn under the cover-glass, their gradual solution may be observed. Other grains are only partly soluble, or are wholly insoluble, in pure water, but these insoluble parts, or grains, can be dissolved by dilute caustic soda (1-2 per cent. solution). Some methods of permanent preparation of stained sections are based upon the fact that the grains can be made insoluble, as, *e.g.*, by treatment for a few hours with alcoholic solution of picric acid, or of corrosive sublimate. This body substance of the grain is bounded externally, and also internally if there be enclosures, by a thin pellicle composed of a more insoluble form of proteiu matter, which is made very visible by the solution of grain and enclosures. The crystalloids are never soluble in water, and on the partial solution of the aleurone grains in this they show up very clearly. They have about the same refractive characters as glycerine, and hence (unless stained) are not usually visible in this or in alcohol as a mountant. They are soluble in dilute caustic potash.

The crystalloids can be well stained with eosin, particularly if the sections have been previously fixed by soaking for at least twelve hours in concentrated alcoholic solution of corrosive sublimate, and the sublimate washed out with alcoholic solution of iodine of a dark sherry-brown colour; but if this is not carefully done needle- or sphere-crystals of the sublimate may be formed. A solution of eosin in absolute alcohol is used and allowed to act for a few minutes. The section can then be put np as a permanent preparation, in Canada balsam, in the manner detailed below, when the ground substance will be seen to be stained dark red, and the protein crystal yellow, while the globoid is nearly colourless.

Logwood (Haematoxylin) added in small quantity to a preparation in glycerine stains the protein crystals and aleurone grains a beautiful violet. The protein crystals come out very beautifully if the section is laid in a drop of 1 per cent. osmic acid; they gradually take on a brownish tint. Or an even better result is obtained by first hardening the sections, and removing the oil, by at least twenty-four hours in alcohol, then placing

them in dilute watery solution of tannin for about ten minutes, and then, after careful washing in water, treating with a 2 per cent. solution of osmic acid. After washing out the osmic acid the preparations may be put up in glycerine or in glycerine-jelly. The crystalloids are permanently stained brown (tannate of albumen). By the same reagent (osmic acid) the oil, if not removed, is slowly blackened—a peculiarity which fat oils have in common with ethereal oils; this reaction is, however, not characteristic, as many other organic substances become blackened by osmic acid. Or, after hardening, place for about an hour in a 25 per cent. watery solution of tannin, wash in water, and then the sections may be placed for an hour in 10-20 per cent. solution of ferrous sulphate, again washed, and mounted in Canada balsam. The crystalloids are then a deep-blue colour.

Mounting in Canada Balsam.—To mount a preparation successfully in Canada balsam requires special preliminary treatment. It must be remembered that no trace of water must be introduced into Canada balsam; hence perfect dehydration of the section becomes necessary. There are usually, indeed, three stages in the method of mounting a preparation in this mountant; dehydration, replacing the dehydrant by some fluid which is perfectly miscible with Canada balsam, and the mounting itself. *Dehydration* is usually effected by alcohol. In the present instance, though the material had been placed for a time in alcohol, stains more or less watery may have been used; it will, however, suffice to place the section for a few minutes in absolute alcohol to secure dehydration; but it will be readily understood that no section coloured with a stain which, like magenta or most of the aniline dyes, is soluble in alcohol, can be treated in this way, for the colour will, of course, be more or less completely dissolved out. Sections stained with these cannot therefore be dehydrated in this (the ordinary) manner. Hereafter one may wish to mount in Canada balsam very watery preparations the direct transfer of which to strong or absolute alcohol may lead to shrinkage or collapse, so that *gradual dehydration* becomes necessary. Various processes are in vogue; a very simple one, and one I [Ed.] have found quite effective, and relatively economical, is to place the sections in a 10 per cent. solution of alcohol, made by adding 4½ c.c. water to ½ c.c. absolute alcohol; then at intervals of a few minutes each removing with a pipette about 1 c.c. of the mixture then adding 1 c.c. absolute alcohol; when you have done

this nine or ten times, the mixture (which will then be about a 90 per cent. alcohol) can be removed with the pipette as completely as possible, and absolute alcohol added. For the sake of economy at each stage up to the last the strongest methylated spirit may be used without any very serious reduction in the percentage strength of the mixture,[1] but it is well then after the complete removal of the last stage of mixture as above to first add pure methylated spirit; then pipette it off and add absolute alcohol. If the dehydration be naturally very complete, as for example with alcohol material dyed with an alcohol stain, the preparation may usually be transferred direct to Canada balsam; usually, however, the *replacement of the dehydrant* is desirable. This is best effected with xylol (or oil of cloves may be used). Xylol is very sensitive to the smallest traces of water, which would make the preparation milky, and hence more or less opaque; previous dehydration must therefore be complete. Milkiness is best observed against a black background, and, if present, the section must again be placed in absolute alcohol, and once more in xylol. Finally, *mounting* consists in transferring the preparation to a solution of Canada balsam in turpentine or in xylol as thick as treacle, and covering with a cover-glass. This will in time set hard; but it is preferable when quite set to run a line of gold size round the edge of the cover-glass, overlapping it as little as possible. This last precaution is quite necessary if at any time an oil-immersion lens is to be used, since Canada balsam is soluble in the immersion oil. Further notes on mounting will be found in Chapter VIII.

Protein-crystals in Brazil-nut.—Protein-crystals of extraordinary beauty, and showing readily all the usual protein reactions, will be found in the oily endosperm of these well-known seeds of *Bertholletia excelsa*. The sections are very easy to make. They should be laid in water, and absolute alcohol run in, when the protein-crystals will show very clearly. The fat oil in this case is not materially affected by alcohol. It also remains unaltered on the addition of acetic acid, while the protein-crystals soon dissolve. In 1 per cent. osmic acid the crystals show up very clearly. These crystals are so large that they can be seen with relatively low magnification, and their form studied. Near the crystal lies a globoid, in this case always in the form of an irregular aggregate of rounded bodies. The ground substance is very rich in fat, and gradually becomes black with the osmic acid. The granular contents of the aleuron-grains also soon become darkly coloured, while the crystals themselves only slowly stain yellow. The crystals are optically uniaxial, and belong to the hexagonal system.

[1] A percentage table will be found in Appendix III.

CHAPTER III.

PROTOPLASM—PROTOPLASMIC MOVEMENTS—THE NUCLEUS—DRAWING WITH THE CAMERA LUCIDA, ETC.—HOW TO CALCULATE MAGNIFICATION—PLASMOLYSIS.

PRINCIPAL MATERIALS USED.

Fresh flowers of *Tradescantia* (best *T. virginica*), just opened. Or *T. zebrina*. Or, very young shoots of *Cucurbita* (Gourd, Pumpkin, Vegetable Marrow, Cucumber, etc.). Or, just opened flowers of *Lamium sp.*

Young fresh roots of *Hydrocharis* (Frogbit). Or, of *Trianea bogotensis*.

Fresh leaves of *Vallisneria spiralis;* or, shoots of *Elodea*.

Fresh young parts of *Nitella*. The same laid for twenty-four hours in 1 per cent. chromic acid.

PRINCIPAL REAGENTS USED.

Potassium nitrate, 10 per cent.—Eosin in water.

Protoplasmic Circulation.—We will now study the phenomena of the movement of living protoplasm,[1] and select as one of the most favourable objects for this purpose the hairs on the staminal filaments of *Tradescantia* (the Spider-worts). *Tradescantia virginica*, and other closely allied species, are cultivated in every botanical and many ordinary gardens, and flower from May or June till late into autumn. The long violet hairs in the flower will at once catch the eye. For observation, select hairs out of a flower which is either on the point of opening or has but just opened. The preparation is made by seizing a tuft of hairs at the base with the forceps; remove them and lay them in water. Or the whole filament can be placed under a cover-glass

[1] For the study of living cells water may sometimes be used as a mounting medium, as in the cases in this chapter; it generally, however, causes pathological changes, and various fluids have therefore been recommended for use. A weak solution (1·0 or 2·0 per cent.) of any neutral salt, *e.g.*, saltpetre (potassium nitrate) may be used, or a dilute solution of sugar (2·0 to 5·0 per cent.), or of gum-arabic.—[ED.

if the anther is previously removed. In this latter case the quantities of air clinging amongst the hairs will give trouble, and it takes some pains to remove them. This is best effected by means of a fine camel-hair brush, with which the hairs are brushed over from below upwards, the tuft being at the same time held firmly at the base. After this the cover-glass is laid on. Most of the hairs will not have suffered, provided the air has been removed with sufficient care.

The hairs in question are formed of numerous barrel-shaped cells, arranged in an unbranched series. At the points of constriction lie the partition walls which separate adjoining cells from one another. Each cell (Fig. 14) shows a thin continuous lining layer of **protoplasm** or **cytoplasm**, and is traversed in the interior by numerous thinner and thicker protoplasmic (cytoplasmic) strands. Suspended within these strands is to be found the **nucleus**, surrounded by an enveloping layer of cytoplasm (shown somewhat below the middle in the figure). The cell cavity in which the nucleus is suspended, and which is traversed by the cytoplasmic strands, is filled by a violet-coloured **cell sap**. It is the **vacuole**. The cytoplasm consists in a colourless, viscous, semi-fluid substance, which is distinguished by the name of **Hyaloplasm** (*i.e.*, clear plasma), and which contains numerous minute granules, known as **Microsomata**, or **Microsomes**. The microsomes have various reactions; some of them are solid bodies, others are vesicular, having a fluid, highly refractive, content surrounded by a skin of plasm. Besides these there can also be seen in the protoplasm, in greater or less number, somewhat larger, highly refractive bodies, which appear somewhat bluish in colour, and which will be designated by the terms **Starch-formers** or **Leucoplasts**. If we focus the microscope upon the cytoplasmic lining layer, it will be seen that this is not in movement as a whole, but rather that fine, net-like, anastomosing, cytoplasmic streams course about within it. In the cytoplasmic threads which traverse the cell-cavity the movement is especially strong. These streams are of varying thickness, they anastomose laterally with one another more or less frequently, and the nucleus manifestly furnishes a central point for them.

FIG. 14. — A cell from the hair on the filament of *Tradescantia virginica* (×240).

Most of the threads end in the plasma layer surrounding the nucleus. The current in any single thread moves often only in one direction; often, however, it can be seen that even in very thin threads there are two currents in opposite directions. The movement is recognisable by the microsomes and leucoplasts borne in the clear hyaloplasm. With continued observation it will be seen that the strands slowly change their thickness and arrangement. New connecting threads form, older ones become gradually thinner in the middle, finally break through, and withdraw into other strands. Thus by degrees the conformation changes. The **nucleus** is almost spherical, or often oval or somewhat flattened. With the strongest magnification which is at our command it appears finely punctate, and in it can be readily distinguished some larger granules (**Nucleoli**). Occasionally two nuclei lie close together in such a cell, because the original

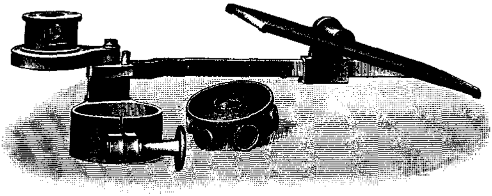


FIG. 15.—Abbé's Drawing Camera, No. 44a (Zeiss); two-thirds natural size.

nucleus has divided. The nucleus is towed about hither and thither by the cytoplasmic strands, and thus slowly changes its position in the cell. In order to prove this, take rapidly a sketch of the cell, and compare this with the arrangement of the nucleus and the threads after the lapse of some time. Accurate sketches can only be taken by means of a drawing prism, and only such have real value for later comparison. We will, therefore, endeavour now to become acquainted with the use of the drawing prism.

Drawing Appliances.—Of all drawing appliances for use in daylight, and not necessitating the removal of the body of the microscope from its vertical position, the most perfect is Abbé's Camera Lucida, manufactured by Zeiss of Jena. It is made in several forms, of which one is illustrated in the adjoining Fig. 15. The apparatus is placed upon the tube of the microscope.

immediately under the top lens of the eye-piece, and clamped there by means of a binding screw, shown in the figure (bottom, left hand). When not in use, the prisms in their sheath are swung back, as shown, so as to be out of the way of the eye in observation. If now we have in proper focus a structure which we wish to draw, the prism sheath is swung forward into place immediately over the eye-piece, and on looking through it in the direction of the eye-piece, we see once more, through camera and eye-piece, the figure of the object in the field of view of the microscope. The reflecting mirror upon its horizontal arm should extend out to the right-hand side of the microscope, and under it should be placed (for drawing) a horizontal drawing-desk, the surface of which should be about ten inches below the level of the eye-piece. Lay a sheet of drawing-paper upon this desk, and rest the point of a lead pencil against it. If the point of the pencil is under the mirror, it should now be visible in the field of view of the microscope at the same time with the figure of the object. The point of the pencil is, however, made visible by double reflection, the first time from the large mirror, the second time from the silvered surface of a small prism in the point of sight of the eye-piece (compare the figure); while the microscopic figure comes directly to the eye through a small gap in the silvering of this prism. If the surface of the drawing-desk does not lie at the distinct visual distance of the observer, the point of the pencil will be seen indistinctly, and the drawing-desk must be raised, or, though seldom, be made lower. We can test the necessary height by means of books laid one upon the other.—The microscopic image and the pencil are only well visible on the drawing surface when a definite relation of brightness exists between the two. The dimming of the paper surface is effected by a cap, to be placed over the camera-prism, and which in the figure is seen lying by the apparatus. Round the rim of this cap are seven apertures, one open, the others closed with disks of neutral tint glass of various degrees of depth, through one or other of which the pencil of light coming from the mirror can be passed. Dimming of the image itself can be effected by the diaphragm, or, in some forms of the instrument, by a wheel of similar disks of glass which can be rotated in the camera-setting itself, just under the prisms. If the arrangement is perfect, you can draw with the lead pencil the outline of the object as if drawing it in the field of view of the microscope.

The second camera (also by Zeiss) is known as the two-prism camera, and is illustrated in Fig. 16, together with a diagram showing the path of the light rays when it is in use. The prism-sheath (K) is fixed upon an adjustable arm (b), and this is mounted by a vertical arm upon a collar which slides under the eye-piece on the end of the tube. This camera has the advantage that it can always be kept on the instrument, the prism-sheath being turned out of the way; and with some practice will perform yeoman's service. It consists of two prisms, inclined to one another, in a common setting. The rays (s) coming from the pencil take, after double reflection inside the prisms, a course parallel to the axis of the microscope, and thus coincide with the rays (s_1) coming direct from the object. The camera is placed in the inclination represented in the figure, and so adjusted that its

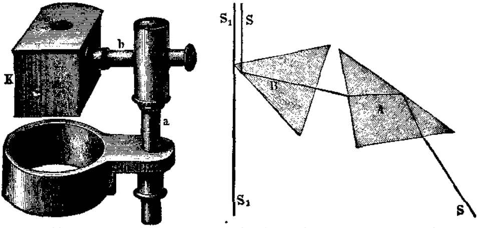


FIG. 16.—Two-prism Drawing Camera by Zeiss; three-quarters natural size.

anterior edge, visible through the opening in the setting, approximately bisects the "pupil" of the emerging rays of the microscope, *i.e.*, the bright circular disk which we notice when we look perpendicularly into the eye-piece from a short distance, such as 1½ inch, above it. If, then, on moving the head to one side we do not see the "pupil" notably displaced towards the edge of the prism, this latter stands also at the right height.—We draw upon a sloping drawing-desk, which is placed in front of the microscope. If, after some attempts, we have found the point of the lead pencil upon the drawing-paper, we can now follow with it the outlines of the object. If the object is not to be distorted in drawing, the drawing-desk must have a correct inclination. In order to determine this rapidly and accurately, we draw the circular outline of the field of view upon the paper

with the aid of our camera, and obtain thus, if the inclination of the drawing-desk is correct, a figure which is likewise a circle; if, on the other hand, we have an ellipse, the slope of the drawing-desk is not correct, and must be varied until a circle is produced. Or, we set in position, and with a high power, the stage micrometer recommended in the Introduction, *i.e.*, a millimeter divided into 100 parts, engraved upon an object-slide. We now turn the stage micrometer round through 90°, so that the engraved lines shall run from side to side, and succeed one another fore and aft. In case the stage is too small to permit such a position of the stage micrometer, we must change the position of the microscope itself 90°. The turning of the microscope naturally renders necessary a readjustment of the mirror. If our instrument is provided with a "concentric rotating stage," or similar appliance, then it is only necessary to turn this; such a stage is very useful for drawing, as it enables us to place the object in the desired position. If we have given the micrometer its proper position, then, with the help of the camera, we draw its lines upon the paper on the drawing-desk. The lines follow one another up the slope of the desk. We shall succeed, without much difficulty, in reproducing it exactly; but, as the lines have a certain thickness, it is necessary that we should represent always a definite edge of the line. The inclination of the drawing-desk is correct when the distance apart of the lines remains the same at all heights. If this distance increases upwards on the desk, the slope must be made steeper; if it sinks, the slope should be lessened. As, however, small errors in our measuring scale are not impossible, it is necessary to represent several parts of it in the same way. In this way we shall find that our desk should have a slope of about 25°. Having once found the correct slope, it is well to have a desk made with its two supporting sides of the correct heights.

When we have obtained the correct inclination of the drawing-desk, this figure, taken from the stage micrometer, can also be used in order to calculate the magnification of the drawing, *i.e.*, the magnifying power of the combination of objective and eyepiece in use. We know already that the lines which we have drawn are 0·01 millimeter (*i.e.*, approximately $\frac{1}{2500}$ inch) apart. If we find that now they lie say 2·4 mm. (*i.e.*, nearly $\frac{1}{10}$ inch) apart, we know that the drawing is enlarged 240 times. This method is

therefore the simplest and best for measuring the size of a microscopical object. If, that is, we have attained the necessary accuracy in drawing, in order to reproduce even slight variations in size with fidelity, and if we know the definite enlargement of the object which we have drawn at exactly the same distance, it needs only to divide the size of the drawing by the known enlargement to get the actual size of the object. If, *e.g.*, one cell of the hair of *Tradescantia* appears, with 240 times enlargement of its figure, to be 9 mm. broad, this indicates an actual breadth of $\frac{9}{240}$ mm., *i.e.*, of 0·0375 mm. This method gives in the simplest way so accurate a result, that in our studies we can confine ourselves to it.

Various other contrivances have been introduced for the purpose of aids to drawing. Some of these, like the Wollaston Camera Lucida, require the body of the instrument to be placed horizontally, and the instrument as a whole to be raised on a pedestal. This can of course only be used with instruments which admit of this position; and for working purposes it is, besides, objectionable in several ways. A very cheap form for use thus is Dr. Beale's neutral-tint reflector, which fixes on the eye-piece, making with its glass an angle of 45°. The student, when he chooses a camera or drawing-prism, should, however, always select one for use with the instrument in the vertical position; and, as he may not improbably obtain one from a maker who is not the maker of his instrument, he should always send the eye-piece of the latter, so that the fittings of the camera may be adjusted to the size of this. Zeiss's camera is adjusted for eye-pieces of the continental size, a size much used now by English makers for their smaller instruments. Of whatever camera is chosen, the method of adjustment upon the eye-piece must be learned from the maker's description (though usually very easy to find out for one's self); the rules laid down above for learning how to draw are equally applicable to all of them. Lastly, the quality of the drawing depends on two factors: the accuracy of the observer, and the skill of the draughtsman. Endeavour to draw accurately what *is*, and do not let your observation be corrupted by forming impressions, or by placing some interpretation upon what you see. If you do this latter, your drawing serves only to illustrate an interpretation which may prove to be wholly inaccurate; if you do the former, your drawing should be consistent with any interpretation. Published figures, particu-

larly in text-books, provide abundant illustrations of the two methods of drawing.

Now turn once more to the cell of the *Tradescantia* hair, and endeavour with one or another drawing apparatus to make a figure of it. As in all ordinary drawing apparatus[1] appliances for the regulation of the light are wanting, so we must endeavour, either by shading the drawing surface, or by changing the position of the mirror, to obtain approximately similar brightness for the drawing surface and the field of view of the microscope. For drawing it is best to use stiff, smooth drawing-cards[2] and black lead pencils. In order that they shall not be effaced, finished drawings can be washed over with very dilute gum-water.

Protoplasmic Circulation, and Plasmolysis.—Take in this way a sketch of the general outline of the cell, of the cytoplasmic streams and the nucleus, and compare it with the same cell after the lapse of an hour, to see whether the form and general arrangement now correspond. As already stated, we shall most probably find that the distribution of the streams has altered, and that the nucleus has changed its position in the cell.

In order to determine that in their streaming the cells are independent of one another, and that the cell-wall does not influence the movement, allow a neutral but water-withdrawing fluid to act upon the hair. From the edge of the cover-glass add to the drop of water a little concentrated sugar solution, or better still, glycerine. Before long the reagent begins to withdraw the water of the cell-sap, and there results a decided contraction of the cytoplasmic sac into the cell. This withdraws from certain parts of the cell-wall. This contraction of the cytoplasmic body of the cell under the influence of dehydrating media is distinguished by the name **Plasmolysis**. It can he then obsetved, that so long as the contraction does not become too strong, the streaming of the cytoplasm still goes on, even in those parts where it has withdrawn from the cell-wall. Soon, however, all movement in the cell is arrested. Yet in most cases to set it going again it suffices to wash out the water-withdrawing reagent by means of water. To this end water should be run under one edge of the cover-glass, while the fluid under the cover-glass is sucked out from the other edge by blotting-paper. The cytoplas-

[1] Excepting such as the best form of the Abbé-Zeiss.—[ED.]

[2] Such of excellent quality and surface are Goodall's thin Bristol Boards. —[ED.]

mic sac then again tends to expand and apply itself to the cell-wall. It not infrequently happens that during the contraction certain portions of the cytoplasm separate themselves from the cell-body, and remain lying against the cell-wall as rounded balls. These balls can also be retaken into the expanding cytoplasmic sac.

It is easy to determine that during the contraction of the contents, observed as above, the colour-material does not diffuse through the living cytoplasmic sac, and that the coloration of the cell-sap becomes proportionally more intense. The appearances in dead cells are quite otherwise. For example, allow absolute alcohol to act upon the hairs; the cytoplasm is immediately killed, and now accumulates colour, for it withdraws from the cell-sap the violet colouring matter, so that this soon appears quite limpid, while the cell-plasm and the nucleus stain deep violet. The violet colour can also pass through the cytoplasmic sac, and diffuse in the surrounding fluid.[1]

The phenomena which are produced when the hairs of *Tradescantia* are laid in a drop of 10 per cent. solution of nitrate of potash, and then taken under observation, are specially interesting. Most of the cells, it is true, show ordinary plasmolysis; but cells will also often be found in which a barely perceptible contraction of the plasmic body has ensued, whereas the cell-cavity, filled with its violet cell-sap, has collected together as an independent structure. In such cases the cell-plasma is quickly killed, with the exception of the layer which surrounds the cell-cavity. This vacuolar membrane is therefore distinguished hy its comparative independence and greater resisting power. At length the cell-cavity forms one or several vacuoles, filled with dark, violet-coloured cell-sap, lying in the disorganised cell-plasma. That the plasmic layer surrounding the cell-sap remains living is proved by its opposing the passage of the colouring matter. If a little watery eosin has been added to the solution of nitrate of potash, the dying plasma, together with the nucleus, is immediately stained red.

[1] Living protoplasm in its *active* condition refuses entrance, therefore, to certain stains, which, when dead, it accumulates. Various aniline colours, such as methyl blue, methyl violet, cyanin, Bismarck brown, magenta, safranin, methyl green, iodine green, and others, are, however, taken into the living cell in a very dilute state. Living protoplasm in its *latent* condition, exactly as when dead, absorbs stains with great avidity, as we saw in staining the section of the pea (p. 29).

If *T. virginica* should not be available, *T. zebrina*, very widely cultivated in greenhouses for hanging baskets, for covering the front of the stage, and as a creeper on the soil under the stage will serve very well, and this will come in very usefully during the winter portion of the year when the former plant cannot be obtained. In summer, on the other hand, *T. zebrina* rarely flowers, though it can be induced to do so in a warm house. In all essentials the staminal hairs of the two plants are alike.

Circulation in Cucurbita Hairs.—Still another very favourable object is provided by the hairs which grow upon the youngest shoots of the genus *Cucurbita* (gourd, pumpkin, vegetable marrow, cucumber, etc.). The preparation is made by removing these hairs at their base by a razor, and placing them in a drop of water on a slide. The stronger hairs are multicellular at the base, and taper off into a row of cells; others bear multicellular heads. The protoplasmic network in the cells is finely developed; it contains microsomes, and, though but sparsely, large, green-coloured **Chlorophyll-grains.** The nucleus is large, suspended by the threads; it has a brightly shining nucleolus, and is carried about hither and thither in the cell. Similar movements to those of *Tradescantia* are shown also by the hairs which stand in two rows on the corolla tube of species of *Lamium* (dead-nettle). We select just opened flowers, which are very easy to find from early spring to late autumn.

Rotation in Hydrocharis.—A very peculiar object is provided by the root-hairs of *Hydrocharis morsus ranæ* (the Frogbit). We select for observation fresh young roots with stiff hairs. The hairs are visible to the naked eye. Cut off an entire root-tip, and quickly place it on the slide in a suitable quantity of water. The cover-glass is laid on in the usual way, the largest cover-glass at our disposal being chosen. Owing to the not inconsiderable thickness of the object, however, all parts will not be accessible with a high power, as the objective will usually come into contact beforehand with the cover-glass. These hair-cells are very long and tubular, and, like all root-hairs, unicellular. The abundant cytoplasm which they contain is in active movement, but here there are not numerous thin streams, forming a network, but a single strong parietal stream, returning upon itself. This kind of movement is called **Rotation,** to distinguish it from the other kind, or **Circulation.** This stream presents the appearance of a broad, slightly spirally wound band, which, if projected upon a

plane, would form a very elongated figure 8. The movement must not, however, be represented as if the band as a connected whole were turned around inside the cell, for, in fact, the neighbouring parts during the movement are continually changing their reciprocal positions. The two streams going in opposite directions are, however, not in immediate juxtaposition, but are separated by a narrow band of protoplasm which is at rest. This "neutral band" or "indifferent band" is reduced to a very thin layer of cytoplasm.

Circulation in Trianea.—In winter, or in other times in the year, when *Hydrocharis* cannot be obtained, it can be replaced by *Trianea bogotensis*, a South American Hydrocharidean, cultivated in most botanical gardens. The form of the root-hairs agrees exactly with that in *Hydrocharis*, as also does the rotation in the fully-developed root-hairs. The young root-hairs, on the other hand, show active circulation, akin to that in *Tradescantia*, with abundantly branched, frequently changing currents. In general, the streams in the lining layer of protoplasm move towards the tip of the hair, and make their way from thence as threads, which traverse the cell-cavity. Between this circulation, and the more restricted rotation, all stages of transition can be observed. The cell-plasma contains microsomes, besides pretty numerous strongly refractive globular bodies, partly perhaps leucoplasts, and vacuoles of different sizes. In the cell-sap are to be seen more or less small stellate agglomerations, probably of calcium oxalate, which are driven about hither and thither by the action of the plasmic streams.

Rotation in Vallisneria.—The leaves of *Vallisneria spiralis* furnish very instructive preparations for illustrating rotation of protoplasm. This plant is found in all botanical gardens, and can be easily cultivated in aquaria in dwelling-rooms. For investigation a strong leaf is selected, and a flat section taken from the lower part of it. For this purpose it answers best to lay the long, narrow leaf across the index-finger, and to hold it down on both sides with the thumb and middle finger. The flat section is taken by moving the razor parallel to the long axis of the leaf. The aim should be to obtain a plate of tissue about half the thickness of the leaf; but if the section should at first sight appear too thick, parts of it which are sufficiently thin for the purpose will probably be found. This section is laid on a slide, epidermis downwards, in a drop of water. Air clinging to it may

make some parts of the section useless, but others will always be found which admit of undisturbed observation. The streaming is not noticeable in the cells of the uninjured plant, but is induced by the stimulus of an injury, and therefore by the preparation of the section. This does not happen immediately, and it may be necessary to wait some little time before strong streaming is visible; it can be best followed in the wide elongated cells which form the internal tissue of the leaf. At a low room-temperature the movement is sluggish, but it can be hastened by slight warming of the microscope slide, as, for example, by laying it for a minute or so on the hand. The stream circles around the entire cell, without, in most cases, to any extent deviating from its direction parallel to the long axis. The "neutral band" is fairly broad, and it is easy in this case to see that the nearer the moving cytoplasm is to the neutral band the slower is its movement, and *vice versâ*. The stream carries with it green coloured chlorophyll grains and the nucleus. The latter is flattened into the form of a disk. From time to time it comes into sight, but as a rule it is concealed by chlorophyll grains. Not infrequently it sticks at a turning point, then the accompanying chlorophyll grains also halt with it, till, an instant later, all again are drawn into the stream. The direction of the streaming changes from cell to cell without any regularity.—If dilute glycerine or 15 or 20 per cent. sugar solution is permitted to act upon the section, the protoplasmic sac can be seen to withdraw from the cell-wall, and the continuance of the streaming at the first moment of contraction can be readily made out, while it is often continued when the cytoplasm has collected into a rounded ball.

Movements in Elodea.—*Vallisneria* may also be replaced, with approximately like results, by the widely-spread river and pond weed, *Elodea canadensis*, the leaves of which are so transparent that they can be used without preparation. The leaf to be studied must, however, be severed from the stem which bears it, for here, as in *Vallisneria*, strong movements are induced by injury. As in *Vallisneria* the cells near the mid-rib are elongated and narrow; they show simple rotation, made evident by the chlorophyll bodies which are hurried around in the current. The shorter and broader cells of the general lamina of *Elodea* may also show us a simple kind of circulation, the vacuole, as in the staminal hairs of *Tradescantia*, being traversed by cytoplasmic threads (often few and coarse) which suspend the nucleus in the

centre of the cell-cavity, while the lining layer shows streams in various directions.

Rotation in Nitella.—The strongest protoplasmic currents known in vegetable cells are met with in the *Characeæ* (Stoneworts). We must, however, take the genus *Nitella*, for *Chara* has completely invested, usually calcified, and therefore opaque, internodes, and it is the internodes which are specially suited for study. For observation we select the younger branches of the plant, and can see at once that the rotating layer of cytoplasm has a considerable thickness. The outer layer of protoplasm immediately lining the cell-wall, in which the chlorophyll grains lie, is motionless. The motionless layer is here, therefore, comparatively thick, while it is in general so thin as to escape observation; for in all the objects which we have already studied an outermost denser portion of the cytoplasmic lining layer, the **Ectoplasm**, takes no part in the movement. An obliquely winding stripe or band on the wall of *Nitella* is free from chlorophyll grains; it attracts the eye by its brightness. This colourless band marks the neutral band in the protoplasmic stream. We have here a repetition of what we found in the root-hairs of *Hydrocharis*, where, however, the neutral band of the cytoplasmic layer was extremely reduced. The internodal cells of *Characeæ* are multinuclear, the protoplasmic current carries with it numerous elongated nuclei, which, however, show up as brighter spots only in the most favourable cases. If the piece of the plant is laid for 12 to 24 hours in 1 per cent. solution of chromic acid, they can often be very readily seen, and their peculiar rod-like, curved, and horse-shoe forms made out. Not to be confused with these nuclei are the rounded balls of apparently albuminoid matter which are seen carried around in the stream in larger or smaller number, and appear either smooth or with a spinous surface. Simultaneously with their movement forwards, these balls are turned upon their axis, which shows that the rapidity of the stream is greatest in that part of its thickness which impinges on the stationary chlorophyll containing outer layer of plasm, and gradually diminishes towards the cell-cavity.—If *Nitella* is laid in exceedingly dilute solution of methylene blue, in a few hours, perhaps not till after a day, these spinous balls are stained blue. As the streaming still goes on uninjured, the blue balls are driven along by its means, and form a very instructive picture.

CHAPTER IV.

CHROMATOPHORES (PIGMENT BODIES)—COLOURED CELL-SAP—LEUCOPLASTS.

Principal Materials Used.

A large-leaved Moss, such as *Funaria hygrometrica* or *Mnium hornum*. The former grows very commonly on ground which has been charred, on limestone walls, etc.; the latter on pots and walls of fern-houses. Or the prothallium of any fern. Fresh.

The same preserved in alcohol.

Flowers of *Tropæolum*, just opened. Or fruits of Hawthorn, Rose, Asparagus, or Tomato.

Flowers of *Strelitzia regina; Verbascum nigrum; Antirrhinum majus.*

Flowers of *Vinca major* or *minor;* petals of Rose; sepals of Larkspur; flowers of *Adonis*. All fresh.

A Carrot.

Leaves of Copper-beech; autumn leaves of Virginian creeper; of Maidenhair tree; of Oak. All fresh.

Pseudo-bulbs of *Phajus grandifolius*. Fresh, or fixed in picric acid and preserved in alcohol. Rhizomes of *Iris germanica;* stems of *Pellioni Daveauana;* fresh.

Principal Reagents Used.

Potash—Dilute chloral hydrate—Methyl violet, gentian violet, or rosaniline violet—0·2 per cent. acid fuchsin.—Fixing reagents: saturated solution of picric acid in water; saturated solution of picric acid and corrosive sublimate in alcohol.

Structure of Chlorophyll Grains.—We have already had an opportunity in several objects of obtaining an insight into the nature and appearance of the **Chlorophyll grains** or bodies; but we will now give some special attention to these structures. We select for this purpose a very widely distributed moss, which is distinguished by very fine, large, lenticular chlorophyll bodies, and of which the leaves, unilamellar with the exception of the mid-rib, permit observation without further preparation. Such

a moss is *Mnium hornum*, or *Funaria hygrometrica*. Numerous chlorophyll bodies of considerable size are to be seen in every cell;[1] in plants which are exposed to diffused daylight they are contiguous only to the free cell-walls; that is, to those which form the upper and under surface of the leaf.[2] Hence they present their broadside to the observer. That they are narrower in profile we see in the isolated grains which underlie the side walls. All stages of division of the chlorophyll bodies are easy to find, and often associated in the same cell (Fig. 17). The resting grains appear quite circular; they then become elliptic, afterwards constricted in the middle so as to be shaped like a figure of eight, and finally completely divided across. The twin grains remain for some time still in contact. The **starch enclosures** of the chlorophyll bodies are, on account of their varying size, in many leaves easy, in others difficult, to see. They are, however, always clearly distinguishable when the chlorophyll bodies get out of an opened cell into the surrounding water, and are there disorganised. With this object we cut a leaf with a sharp pair of scissors into several pieces. The starch grains, set free from the disorganised chlorophyll bodies, augment in size, and are identified as such with iodine. On the other hand an entire uninjured chlorophyll body is coloured murky brown with iodine, from a combination of the blue coloration of the starch enclosures, the yellowish-brown coloration of the protoplasmic ground substance, and the green of the chlorophyll. In order to obtain favourable iodine coloration of the uninjured chlorophyll bodies, we take for study leaves which have lain some time in alcohol, and are thereby decolorised. The chlorophyll bodies now appear colourless; their starch enclosures become coloured, by gradual action of the iodine solution, more quickly than the protoplasmic body. The iodine reaction is still more

FIG. 17.—Chlorophyll bodies from the leaf of *Funaria hygrometrica*, resting and in division.

[1] The cell-walls and cytoplasm of this leaf are sufficiently resistant to permit the preparation to be put up in water without the chromatophores suffering. In many cases, however, they are injured by water, and it has been recommended then to put the preparation in pretty fresh olive oil, in which the cells not merely remain alive for a long time, but the oil serves to expel the air from the intercellular spaces, and, from its refractive properties, increases the transparency of the preparation. A dilute solution of a neutral salt, or of sugar, as advised for living cells, may also be used.—[ED.]

[2] This is known as the position of *Epistrophe*.

noticeable if the preparation is previously treated with potash, which causes the starch grains to swell. This last method also enables the smallest quantity of starch in the chlorophyll bodies to be recognised; but the result is even more certain if the chlorophyll grains are treated with a solution of five parts of chloral hydrate in two parts of water to which a little iodine solution has been added on the object-slide. The chlorophyll is dissolved, so that in a few minutes the leaf appears colourless; simultaneously the chlorophyll body swells, and also the starch grains which it contains, and these last come out clearly with the blue colour.—Leaves decolorised with alcohol show also very beautifully, with the same treatment, the blue-stained starch grains in the chlorophyll bodies, while these last are not stained. After the chlorophyll bodies have been decolorised by alcohol they can be stained also very well with very dilute watery solution of methyl violet or of gentiana violet. The cell membranes also are coloured, but the chlorophyll hodies more deeply, and therefore stand out more sharply. Rosaniline violet also is an excellent stain.

With stronger magnification the living chlorophyll bodies of the leaf of *Funaria* appear to be finely porous; this can also be seen in other chlorophyll bodies, such as those of Vallisneria, and very well indeed in Crassulaceous plants of the common cultivated genus *Echeveria*, in the uninjured cells of the lower mesophyll cut parallel to the surface.

The same results as with leaves of *Funaria* are obtained with prothallia of ferns, so that the two objects can replace one another. Prothallia are always readily to be found in plant houses in which ferns are cultivated; and any species can be used.

Change of Position of Chlorophyll Grains.—The same moss *Funaria*, or *Mnium*, can be used for the purpose of a physiological demonstration under the microscope, to the effect that the position of the chlorophyll bodies in the cells is subject to the influence of light. Both of these plants are very sensitive to light, and the leaves can be examined entire. Plants which have for a considerable time been exposed to diffused daylight show the chlorophyll bodies collected on the upper and under surfaces of the leaf-cells (position of Epistrophe); if removed into a dark room, or placed under an opaque shade, usually a few hours, but sometimes one only, suffices to show the chlorophyll bodies re-

moved to the side walls of the cells, leaving the upper and under walls free (position of Apostrophe). The former is the normal position in daylight, the latter at night. A short time, often less than an hour, in diffused light, suffices to change the position in the previously darkened plants from that of Apostrophe to that of Epistrophe. In the latter position the chlorophyll bodies, which, as already seen, are lenticular, present their broad sides to the light, and therefore assume the best position for effective assimilation. If a strong tuft of the moss is placed in bright sunshine, and protected from too high temperature by a water screen, the chlorophyll bodies again pass to the side walls.

Orange Chromatophores of Tropæolum.—In order to become acquainted with chromatophores of other colours, let us turn next to *Tropæolum majus*, the so-called "Nasturtium" of gardens. We select for study flowers which have only just opened, because in older flowers the colour-bodies begin to be disorganised. Let us first take surface sections from the upper side of the sepals. The preparation can also be made with a fine pair of forceps, if these are stuck pretty deeply into the tissue, and a strip torn off. The preparation is laid in a drop of water, with the epidermal side upwards. Examine it at once, because the injurious action of water on the colour-body quickly makes itself felt. The margin of the section will suffer immediately, so that cells that are still unchanged should be selected for more careful examination. The chromatophores are yellow, shading into orange-red. They appear spindle-shaped, three or four angled (Fig. 18), in forms which border on, and in fact are, crystalline. When unchanged they are homogeneous; under the influence of water they swell, become rounded off, and vacuolate; that is, hollows filled with water appear in their interior. They overlie in especial number the inner wall of the upper epidermal cells.—The brown stripes on the upper side of the sepals are due, as similar sections show, to bands of epidermal cells which are filled with carmine-red cell-sap. These cells contain also yellow grains, which, however, the coloured cell-sap makes almost invisible. In the red cells

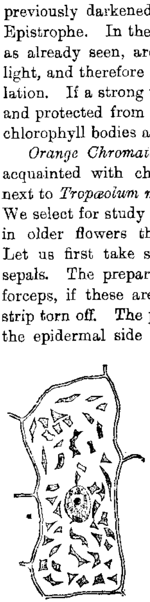


FIG. 18.—From the upper side of the calyx of *Tropæolum majus*. The inner wall of an epidermal cell with the colour-bodies (chromatophores) adjacent to it (× 540).

the nucleus shows usually as a clear spot.—The petals show similar relations; here the edges of the limb, as well as the cilia (or fringe-like appendages) at the base of it, can be used for observation in their entire thickness. The air adhering to the limb hinders observation, but spots free from air will always be found, or can be freed by light pressure. The sepals, however, are always preferable, since the papillæ hinder observation in the petals; for it is evident that, with the exception of the brown stripes on the two lower petals, each epidermal cell of the upper and under side is prolonged in its centre into a blunt cone, one of the papillæ in question. These papillæ are more strongly developed on the upper than on the under side of the petals, and give to them a velvety appearance. The air is entangled very strongly between them. The fiery-red spots at the base of the petals are due to rosy cell-sap and yellow granules in the epidermal cells.—During the investigation it will have been noticed that the surface of the epidermal cells of the upper side of the sepals is longitudinally striate. The striæ are not interrupted at the limits of the individual cells, and are folds of the cuticle which covers the epidermis.—With watery solution of iodine the colour-bodies can be fixed pretty well, and take on at the same time a green coloration; they are very sharply defined. The nucleus is at the same time coloured yellowish-brown, its nucleolus becoming very visible. With methyl violet, rosaniline violet, or with gentian violet the colour-bodies are coloured violet.

Similar chromatophores to those of the flowers of *Tropæolum* can be found in autumn and winter in the coloured flesh of the fruit of the hawthorn (*Cratægus*), the hips of the rose, and the berries of the asparagus; while the comparatively large orange-red chromatophores in the fruit of the tomato (*Lycopersicum esculentum*) show the form of chlorophyll bodies.

If the large and beautiful flowers of *Strelitzia regina*, frequently grown under glass in botanical gardens, are at our disposal, opportunity of examining them should not be lost. In the cells of the orange-red coloured perianth-segments will be found elongated spindles of (for chromatophores) quite exceptional size, and which are, therefore, proportionally easy to examine.

Verbascum nigrum.—Yellow or orange-red colouring matter is almost always combined with a protoplasmic basis; but isolated cases occur where it is met with dissolved in the cell-

sap. Let us fix our attention more closely on such a case in the common mullein, *Verbascum nigrum.* We can examine the petals in water without special preparation, but must remove the adhering air, even if only partially, either by pressure or under the air pump. The epidermal cells of both upper and under side have undulating (sinuous) outlines; the yellow colour of their cell-sap is at once noticeable. The brown spots at the base of the petals are due to a cell-sap coloured from purplish to brown. In the epidermis of the staminal filaments, from which lamellæ can be easily cut with the razor, we see a yellow sap; but besides this there are also in each cell a cinnaber-red irregular lump of colour-material, and a number of colourless leucoplasts filled with starch grains.

Antirrhinum majus.—Similarly it can he at once determined that the yellow-coloured parts of the lower lips of the corolla of *Antirrhinum majus* (the Snapdragon) contain a sulphur-yellow sap in their cells; the parts coloured red have a rosy cell-sap, and here and there one, seldom more, carmine-red ball of colour-material.

Coloured Cell-sap.—Blue and red petals almost always owe their hue to a coloured cell-sap. A blue cell-sap is found in the epidermis of the corolla of the Periwinkle, *Vinca major* or *V. minor.* The epidermis of either side can be readily torn off with the forceps. The epidermal cells, especially of the upper side, are swollen out into papillæ. The side walls of the epidermal cells show ridges projecting into the cell cavity (Fig. 19), often swollen at their inner edges, so that they may even spread out into a T-form, and, on account of the stronger refraction of their outer surface and the weaker refraction in the interior, quite give the impression of folds. Upon bent portions of the edge of the petal it can be easily seen that these ridges extend the full height of the cell. [Similar ridges will be found in the mesophyll of the leaf of *Pinus.*]

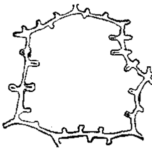


FIG. 19.—An epidermal cell from the under side of the petal of *Vinca minor* (× 540).

Red cell-sap can be seen in the petal of a rose. Here also the epidermis can be readily removed from either side. The upper side has fairly strongly developed papillæ, and hence appears so beautifully velvety. The cuticle exhibits strongly marked striation

In the blue sepals of the Larkspur, *Delphinium consolida*, we find the epidermis of both upper and under sides composed of cells with sinuous outlines, and the epidermal cells of the upper side are also protruded, each into a papilla. The cuticular striations ascend the papillæ on all sides, so that by focussing the microscope at the mid-height of the papillæ, star-like figures arise. The cells contain a blue cell-sap, somewhat shading into violet, besides also, in many cells, blue stars, which consist of short needles of crystallised colour-substance. The epidermis can be removed in small pieces; moreover, the sepal is sufficiently transparent, after removal of the air, to permit examination at the edges through its entire thickness.

Examples of blue and red cell-sap can be easily multiplied, for they are found respectively in almost all blue and red flowers. The case of the bright red flowers of *Adonis aestivalis* and *A. autumnalis* becomes, therefore, so much the more remarkable. The preparation can be removed with the forceps, and in the epidermis we see beautiful, nearly round to elliptic, red grains, comparatively large, and attaining the size of chlorophyll bodies. They appear finely granular, and in water separate quickly into very small grannles, which show molecular movements ("Brownian movement"). The epidermal cells are elongated; their cuticle longitudinally striate; the striæ are clearly continued over the limits of the cells. The same phenomena are shown by the cells of the red perianths of species of *Aloe*.

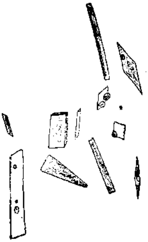


FIG. 20.—Colour-bodies from the root of the Carrot, some with starch grains (× 540).

Colour-bodies of Carrot.—The root of the Carrot, *Daucus Carota*, furnishes a very interesting object. The orange-red colour of this root arises from carmine and orange-red colour-bodies of a crystalline form. The most common shapes are found collected in Fig. 20. They are small rectangular plates or rhombs, the rhombs often acicularly elongated, and prisms of different lengths, often broadening out fan-wise at one end. Such crystalline formations have often small starch grains projecting from one side; these crystalline structures must therefore be placed in the same category with chlorophyll and other colour-bodies as starch-forming plastids (amyloplasts). The crystallised colour-material,

however, is what in this case determines the form, only a small quantity of protoplasm adhering to it, from which the starch grains arise.

Coloured Leaves.—If we examine also one of the coloured varieties of our shrubs or trees, such as the copper-beech, or else an herbaceous plant with leaves coloured reddish-brown, such as the beetroot, we see that the cells of the epidermis contain a rosy cell-sap, and that therefore the joint action of the red of the surface and the green of the interior gives the reddish-brown compound colour.

Autumnal Colours.—In the autumnal coloration of the leaves of the Virginian creeper, *Ampelopsis hederacea,* we can determine that the rose-coloured cell-sap arises in the cells of the internal tissue, and not of the epidermis. The yellow autumn tints of leaves depend on the yellow colour of the disorganised chlorophyll bodies, as is shown in the most beautiful way in the leaves of the Maidenhair tree, *Ginkgo biloba* (*Salisburia adiantifolia*), or, failing this, those of the various species of Maple. Brown autumn tints of leaves may arise from a corresponding staining of the cell-walls, but chiefly, however, from the cell-contents, as is easy to determine in the case of the Oak.

Origin of Starch.—Starch grains are found in certain specialised protoplasmic structures. We have already recognised chlorophyll bodies in this capacity, also certain other colour-bodies in which starch grains are often present; and have also made reference to colourless starch formers. Upon these last devolves the formation of starch in the deeper layers of the body of the plant. We can comprise *all* three structures under the name of **Chromatophores**, and distinguish chlorophyll bodies, colour-bodies, and colourless starch-formers as **Chloroplasts, Chromoplasts**, and **Leucoplasts** respectively. These structures have similar origins, and can be transformed into one another. They are living constituents of the protoplasm of the cell, and lie embedded in the cytoplasm. On the other hand the blue stars, which we found in the cell-sap of *Delphinium consolida,* only represent colour-material crystallised out from the cell-sap, and are, like the lumps of colour-material which we found in the red cell-sap of *Verbascum nigrum,* not to be reckoned as chromatophores, which latter, are essentially *protoplasmic bodies impregnated with colour-material.*

Leucoplasts of Phajus.—The largest and most beautiful starch

grains are produced by leucoplasts, but such leucoplasts are not usually easy to see. About the most favourable material, if it can be obtained, is the comparatively young pseudo-bulb of one of the tropical orchids very widely cultivated in glass-houses for decorative purposes, *viz.*, *Phajus grandifolius.* We halve a pseudo-bulb, make thin *longitudinal radial sections* from its apical portion, and rapidly transfer for examination to normal iodine solution diluted with half its volume of distilled water. If we turn our attention to the deeper portions, which have not been injured in cutting, we shall find without difficulty, at the base of the large and very eccentric starch grains, the flat elliptically elongated colourless leucoplasts (Fig. 21 *B*), appearing rod-like in profile (Fig. 21 *A*). Each leucoplast encloses a rod-like protein crystal, or crystalloid. Towards the surface of the green pseudo-bulb the leucoplasts begin to be coloured green, and take gradually the colour and ordinary appearance of chlorophyll bodies. This material can be cut up small and fixed in concentrated picric acid for about twelve hours, carefully washed in water to remove all the acid, and preserved in alcohol.

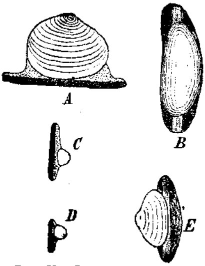


FIG. 21.—Leucoplasts of *Phajus grandifolius*, from the pseudo-bulb. *A*, *C*, *D* and *E*, lateral views; *B*, from above; *C* and *D*, with the starch grains still quite small; *E*, coloured green (× 540).

Leucoplasts in Iris.—A moderately favourable object, and one not difficult to obtain, is furnished by the rhizome of *Iris germanica*. *Surface-sections* of this are made parallel with the surface of the rhizome. The outermost layer of tissue is rejected, and to this follow the starch layers. The observation is in this case best made in water. In uninjured cells the leucoplasts appear as aggregations of protoplasm at the hinder end of the starch grain (Fig. 22). These latter increase only at this end, and have a proportionally eccentric structure. The leucoplasts while under observation rapidly become granular, and separate at length into smaller grains, which show molecular movement—"Brownian movement". Two starch

FIG. 22.—Starch formers with starch grains from the rhizome of *Iris germanica* (× 540).

grains on one leucoplast occur not infrequently. After further growth such grains presently come into mutual contact, and may receive thenceforth layers which are common to the two. These and similar phenomena lead, here and in other cases, to the formation of compound or semi-compound starch grains.

Starch Formers in Pellionia.—A plant which has starch formers quite similar to those of *Iris*, but much more easy to see, is the pretty Urticaceous hot-house creeper, *Pellionia Daveauana*. The plant can be very readily cultivated, and multiplies and spreads freely. The starch formers can be found in every *cross-section* taken through quite young parts of the stem. They are here green, and therefore in this respect are not true leucoplasts, hut in their function are so far related to leucoplasts that they form the greater portion of their starch, not through their own assimilation, but from migrated reserve food-materials. The circumstance that they are green makes them readily visible; a slime with which the section becomes covered protects them from the injurious action of the water. Here also the starch grains which are produced by the starch formers become very large, and show a strongly eccentric structure. At the end of the starch grain most remote from the organic centre, the starch former is still to be seen as a green cap.

Fixing and Staining Chromatophores.—It is usually somewhat difficult to "fix" chromatophores in an nnaltered condition. The best medium for this purpose is a saturated solution of picric acid and corrosive sublimate in alcohol. The action of this must be continned for about twenty-four hours, and the material then well washed in running water. Treated in this way the material does not usually show the formation of disturbing needle-shaped or sphere-crystals of corrosive sublimate; but if the washing is imperfect they may be subsequently removed by running through alcoholic solution of iodine of a dark-brown colour.[1] In staining the chromatophores acid fuchsin is recommended, made by dissolving 0·2 per cent. acid fuchsin in distilled water, and a little camphor added to make the solution keep. The sections can lie

[1] Objects fixed in corrosive sublimate should not be touched with steel forceps or section-lifters before washing, or globules of metallic mercury may be produced. If forceps must be used they should have platinum or horn tips.

in this for several, even up to as many as twenty-four, hours. They are then washed as rapidly as possible in running water and can be examined in glycerine, or dried and mounted in Canada balsam. They must not, however, be treated with alcohol, as the stained chromatophores would be decolorised.

CHAPTER V.

TISSUES — REACTIONS FOR CELLULOSE, SUGAR, INULINE, NITRATES, TANNIN, LIGNIN—MINERAL BODIES—THICKENING OF THE CELL-WALL—PITS.

PRINCIPAL MATERIALS USED.

Beetroot, white preferred ; fresh.
Unripe Pear.
Tubers of Dahlia ; fresh ; also in alcohol.
Infloresecense of *Euphorbia helioscopia ;* in alcohol.
Oak-galls, fresh or dried.
Vinca major ; stems ; fresh (or in alcohol).
Seeds of *Ornithogalum.* Or twigs of Cherry-laurel.
Date stones.
Old stem of *Pinus sylvestris ;* dry or in alcohol. Also fresh twigs.

PRINCIPAL REAGENTS USED.

Dilute acetic acid—Sulphuric acid, 50 per cent., 66 per cent., and pure—Strong hydrochloric acid—Aqueous logwood—Chlorzinc iodine—Fehling's solution—Diphenylâmine (0·05 gram in 10 c.c. sulphuric acid)—Orcin in alcohol—Phloroglucin—Iron chloride—Potassium bichromate (10 per cent. in water)—Concentrated solution of molybdate of ammonia in concentrated ammonium chloride—Acetate of copper—Ammoniacal copper-oxide—Concentrated solution of chromic acid—Aniline sulphate—Eau-de-Javelle.

Tissues of Beetroot.—We commence with the white Sugar-beetroot (*Beta vulgaris*).[1] A small piece of tissue is taken from the fleshy root, and from this is made a microscopical preparation. We choose for examination a *radial longitudinal section, i.e.*, a section which is taken parallel to the long axis, and in the direction of the radius. This section cuts at right angles the concentric rings of the root, which are visible to the naked eye. Examined

[1] Red beetroot can be used, though not so good, and the parts of the section from which the red cell-sap has disappeared will be of most value.—[ED.]

in water, this section shows us more or less rectangular **cells**, filled with a watery, colourless fluid. On the walls of these cells we notice, here and there, larger or smaller, brighter, round or oval spots, which indicate shallow **pits**, *i.e.*, local thin places, or hollows, in the wall. In some of the cells the nucleus is visible, with a distinct nucleolus, and surrounded by minute leucoplasts; also a layer of cytoplasm lining the cell-wall. The **intercellular spaces** are usually filled with air, appearing black. In certain parts of the preparation the parenchymatous cells are narrower, and elongated parallel to the long axis of the root; between them are visible long tubes usually filled with air, which are distinguished by a characteristic thickening of their walls. These tubes are **vessels**. The thickening of their walls is in a network of pits ("reticulated"); that is, the wall shows thickening bands combined into a network, between which lie unthickened places. These unthickened places, or pits, are elongated transversely to the longitudinal direction of the vessels. Where the section has opened a vessel, there can be seen in it, from time to time, ring-like thickenings, which project into the interior of the cells. These are the diaphragm-like remains of originally complete partition walls, and from these remains it will be seen that the vessel has proceeded from the coalescense of a row of cells. The air present in the vessels often interferes with examination; it can be extracted with the air-pump. When an air-pump is not at our disposal, we can endeavour to remove the air by laying the preparation in freshly boiled but cold water. This is more quickly done by a short immersion of the preparation in alcohol, which, it is true, kills the contents of the cells, but for the foregoing observation this is not of consequence.

Here and there also in the preparation we come across isolated cells, which are densely filled with small clinorhombic **crystals**, and appear almost black. These crystals consist of oxalate of lime. In order to prove this, we allow dilute acetic acid to act upon them, and determine that they are insoluble in it. Into another preparation we run sulphuric acid, and the crystals are quickly dissolved without evolution of gas. The quantity of sulphate of lime (gypsum) formed is so small that it remains dissolved in the surrounding fluid.[1]

[1] *Calcium Oxalate Crystals.*—In cases hereafter, calcium oxalate crystals may be found in quantity, so that upon treatment for a few minutes with

Reactions of Cellulose.—The structural relations in the cells of the beetroot show up still more beautifully and distinctly if the section is treated for some time in a watch-glass with a solution of logwood diluted with water. All the cell-walls are then coloured violet, excepting the strongly lignified walls of the vessels. *On the parenchyma-cells the pit-surfaces are either not stained* or else show a slightly stained network. Unlignified membranes which are stained with logwood owe this coloration to the **pectin** with which the cellulose is mingled. The nucleus also stains pretty deeply. The vessels contain neither nuclei nor plasmic contents.—If a section is laid in chlorzinc iodine the characteristic violet **cellulose-reaction** soon appears. The colour begins at the edges of the section, and progresses slowly inwards, but is often not complete for hours; the lignified thickening layers of the vessels stain yellow-brown. If we are dealing with alcohol material soaking in water will hasten the reaction. On the walls of the cells, the surfaces of the pits once more remain unstained,

sulphuric acid (best, dilute 50 per cent.), the resulting sulphate of lime, instead of being dissolved, may crystallise out in the form, usually, of fine needles. These will probably be found spread through or over many cells, surrounding the original crystallogenous cell; but if it be desired to change oxalate into sulphate of lime *in situ* it can be done by heating the section up to boiling-point in dilute sulphuric acid (2 acid, 1 water). The resulting masses of gypsum crystals often occupy the same space and form as the original calcium oxalate crystal. This is particularly striking where the crystals have a well-marked form, as, *e.g.*, the quadrate octohedra in the stem and petiole of many species of Begonia.

Hydrochloric acid has also been much used as a solvent of oxalate of lime without evolution of gas, but the large crystalline masses in which the salt is usually found are as a rule embedded in a mucilaginous jelly, which retards the action of the acid, though it can be hastened by warming.

Calcium oxalate crystals are very doubly refractive, and hence show up beautifully under the polariscope, glistening brilliantly upon the dark background when the prisms are crossed by rotation.

Calcium carbonate, occasionally met with in the form of a deposit in the cell, as, *e.g.*, the large *cystoliths* in special epidermal cells of the india-rubber plant (*Ficus elastica*), but more commonly as a deposit in the interior of cell-walls, or as an external crust to the plant (many water plants and many Saxifrages, *e.g.*, outside the water-pores of *S. crustata*) is best tested by its ready solubility in dilute, not concentrated, acetic acid, with evolution of bubbles of gas, carbonic acid gas; or concentrated, not dilute, hydrochloric acid may be used with similar results. Sulphuric acid forms gypsum crystals, as with the oxalate.

All these reactions are best performed with micro-preparations.—[Ed.]

and stand out specially distinctly. These pit-surfaces are always rounded, of variable size, and irregularly distributed, singly or in groups. Large pit-surfaces are generally traversed by violet striæ of various breadth; they are formed into compartments by them, and give the impression of an irregular lattice. Bright granules, coloured yellow-brown by the chlorzinc iodine, adhere in larger or smaller quantity to the pit-surfaces. This violet coloration with chlorzinc iodine is a specific, though often slow, reaction for cellulose, which is not due to the pectin materials, and ceases when the cellulose has been previously removed from the cell-walls by prolonged action of ammonium cupric-oxide. (As to the action of this reagent see *postea*, p. 72).

For the purpose of comparison we now try the cellulose reaction with iodine and sulphuric acid. The section is first impregnated with iodine solution, best with potassium-iodide iodine solution (1 per cent. iodine and 1½ per cent. potassium iodide), and afterwards transferred to dilute sulphuric acid (2 volumes acid to 1 volume water). The reaction commences at once, from the edges inwards; the section takes a beautiful blue colour. The lesser pits here also remain uncoloured; the larger ones appear latticed with blue.

Sclerenchyma in the Pear.—We now prepare a section from a ripening Pear. The pulpy flesh of the fruit appears here also to be composed of regular thin-walled parenchyma, of large cells, more or less rounded at the angles. These cells contain colourless cell-sap, a very reduced plasma-sac, and a nucleus. Scattered in the tissue are found nests of strongly-thickened cells (Fig. 23). The number of the "stone cells" thus grouped varies from part to part, according to the kind of pear; they form the so-called "grit" of the pear. The cells are distinguished by the notable thickness of their walls, and by the numerous, fine, branched deep pits or pore-canals. Their branching arises from the diminution in the number of the pore-canals as the cavity of the cell is reduced by the great increase in thickness of its walls, so that several open into the cell-cavity by a common canal. Where two thickened cells are in contact, it can be determined that the pore-canals coincide in position with one another. In their fully developed condition, in which we see them here, these cells no longer contain living cell-contents, but only a watery fluid. They represent, therefore, only dead cell-cases, or cell-skeletons (exo-skeletons). After treatment with chlorzinc

iodine, the thin parenchyma-cells take on gradually, but even more slowly than in the beetroot, a violet colour; the strongly thickened cells rapidly become yellow-brown. These latter are therefore lignified, and belong, on account of their strong thickening and lignification, to the **sclerenchyma** or *mechanical* **tissue.** The structural characters of the thickened cells become especially clear under treatment with chlor-zinc iodine.

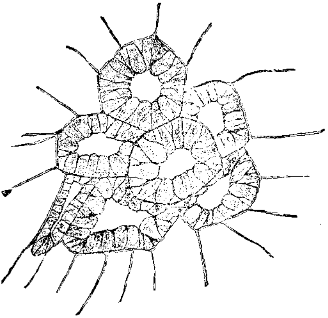


FIG. 23.—From the flesh of the fruit of the Pear. Strongly-thickened cells with branched pore-canals, surrounded by thin-walled parenchyma (× 240).

Reactions of Sugars.—We will use the flesh of the pear in order to test the micro-chemical reactions for **sugar.** Most commonly used is that obtained with Fehling's solution. For this purpose we prepare three solutions, of which one contains 35 grammes of sulphate of copper, the second 173 grammes of potassio-sodic tartrate (Rochelle salt), the third 120 grammes caustic soda, each dissolved in a litre of water; or smaller quantities in like proportions. One volume of each of these solutions must be mixed, for use, with two volumos of water. The separate solutions can be kept ready prepared, while when mixed they in time undergo change. The reaction can be carried out upon an object-slide. For this purpose we place upon it a large drop of distilled water, and three small drops of the ready-made solutions, and mix them with a glass rod. The section on which the reaction is to be tried must not be too thin, should contain at least two layers of uninjured cells, and naturally should not previously have been laid in water. The sectiou should not therefore be cut till the Fehling's solution is ready mixed, and should then be laid immediately in it and covered with a cover-glass. The object-slide can then be warmed over a spirit lamp or

gas flame, till small bubbles commence to form in the fluid. By this time the section will have become a beautiful vermilion-red colour from the presence of reduced copper protoxide, which can be seen in the cells under the microscope in the form of very numerous grains. There is present in the cells of the pear, therefore, a substance which reduces the alkaline copper oxide solution, a substance belonging to the grape-sugar group (**glucoses**), in this particular case **grape-sugar** or **lævulose.**

For comparison we repeat the experiment with a section of beetroot.[1] This contains, as is well known, a member of the cane-sugar group, *viz.*, **Cane-sugar.** Heated in the same way in Fehling's solution this shows no precipitate in the cells; under the microscope the section has a blue colour. If the section is boiled for a longer time in the solution it begins to be coloured vermilion-red on the surface; the cane-sugar is inverted, *i.e.*, converted into glucose, by the strongly alkaline reagent, and now gives the protoxide precipitate. Under the microscope the peripheral cell-layer now shows the vermilion-red grains, while, provided the action has not been too prolonged, the inner cells still show the blue colour due to the presence of cane-sugar.

Reaction for Nitrates.—We will once more use the Beetroot, in order to realise the micro-chemical reactions for **nitrates** by means of diphenylamine. This reagent, used by the chemist for the detection of very small quantities of nitrates and nitrites, serves excellently for histological purposes. We prepare *cross* or *longitudinal sections* through the beetroot, taking care, however, that the sections extend to the surface. We can, with advantage, allow the sections to previously become somewhat dry on the object-slide, and then add the reagent. We use 0·05 gram. diphenylamine in 10 c.cm. pure sulphuric acid. Immediately after the addition of this a deep blue coloration (formation of auiline blue), shows in the outermost zone of the section. This zone contains the youngest tissue of the root, still in course of development; it is this, therefore, which contains the nitrate. From these parts the colour quickly flows over the rest of the preparation, but in the first moment of the reaction the coloured zone is often sharply delimited. Nitrites, and various other salts, appear to give the same reactions, but ash analyses tend to show

[1] Red beetroot can be freely used for this reaction, as the red colour disappears at once with action of the reagent. Or the parsnip may be used in its stead.—[ED.]

that nitrous salts do not occur in the living plant, and as the other salts in question are at least very rare, we can fairly conclude that the reaction indicates the presence of a nitrate.—If instead of the somewhat dried section a fresh one is used for the reaction, the colour which is formed is diffused far more rapidly in the surrounding tissue, and the coloured zone is less sharply delimited. If quite dry material is used, the reagent should be in the form of a concentrated solution in concentrated sulphuric acid.—While the diphenylamine reaction appears in such a characteristic fashion in the sugar-beet, in other plants it may *fail, although nitrates are present; so that if it fails we are not* justified in drawing a final conclusion as to the absence of nitrates; it is a positive, but not a negative, test.

Structure of Cell-wall in Dahlia.—As the next object of investigation we take the tubers of the Dahlia (*D. variabilis*). In the tuber, halved longitudinally, we can readily recognise the central pith. A *longitudinal section* prepared from this shows under the microscope more or less rectangular cells, arranged in longitudinal rows (Fig. 24) having a very reduced cytoplasmic sac, with nucleus, and colourless cell-sap. The intercellular spaces are filled with air; the cell-walls finely striate. The *striæ* are oblique, to the extent of from 35° to 40°. We appear to see two diagonally opposed systems of striæ in the same plane; this is explained by the comparatively small thickness of the walls. In fact, the two opposing systems of striæ belong to the walls respectively of two juxtaposed cells, as can be determined especially at the free edges of the section. With chlor-zinc iodine the *striæ soon colour violet; the intermediate places*, where broad enough to see clearly, are manifestly uncoloured.—A *cross-section* of the same tissue shows on the end walls of the cells, which we then see in surface view, no appearance of striation. It shows only here and there larger rounded pits; hence the neces-

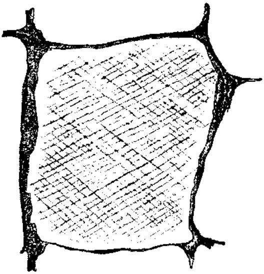


FIG. 24.—From the pith of *Dahlia variabilis* (× 240).

sity for cutting longitudinal sections to show the striate structure. It is a widely-spread phenomenon that the cross walls in a tissue are thickened differently to the longitudinal walls—a phenomenon probably associated with the differing demands on their permeability for the conduction of food-materials, etc.

Inuline.—If the section is laid in absolute alcohol there arises in the cell-sap a fine precipitate of **Inuline.** Replace the alcohol by water, and warm the object-slide over a spirit flame, and the precipitate is again dissolved.—In order to study the inuline in the shape of **sphæro-crystals,** it is best to examine pieces of tubers which have been placed in about 50 per cent. alcohol at least eight days before. We examine the section best in water, and during the examination allow nitric acid very slowly to enter. The sphæro-crystals or sphærites (Fig. 25) are always found on the cell-walls, forming more or less perfect balls. The ball can be traversed by cell-walls. Usually several variously sized balls form together a larger group. The balls allow a radial structure to be more or less clearly recognised; this structure comes out more sharply when the nitric acid begins to work; it arises from radially arranged needle-shaped **(acicular) crystals,** which compose the ball. In the growing sphærite they are deposited in concentric layers, whence arises its concentric lamination. Iodine solution produces no coloration. If the sphæro-crystals are warmed in a drop of water on the object-slide they quickly dissolve.—For the recognition of inuline in solution in the tissues an alcoholic solution of orcin can be used. If sections are saturated with this, and then boiled in hydrochloric acid, a deep orange-red colour is produced if inuline be present. Any sphærites of inuline which may be present are, of course, dissolved in the process. Phloroglucin can be used instead of orcin, with a

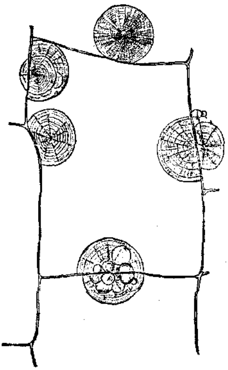


FIG. 25.—From the tuber of *Dahlia variabilis*, after lying in spirits for many months. Sphæro-crystals on the walls (× 240).

resulting brownish colour.—With polarised light the sphærites of inuline show a black Maltese cross on a bright ground, just as do starch grains.

Calcium Phosphate.—The sphærites of dahlia tubers are not always of inuline. In tubers preserved in alcohol, bulky sphæro-crystals of calcium phosphate are often deposited. They have the same form, but usually smaller size than those of inuline, and are slowly dissolved in water if the preparation be examined therein, their contour remaining to the last, while their refractive power continually decreases. The sphærites of calcium phosphate are either without clear lamination and distinct nucleus, or they show an amorphous nucleus, which is surrounded by a shell of acicular crystals. In the latter form the nucleus of the sphæro-crystal is deeply stained by carmine solution; in the former the sphæro-crystal is stained throughout its entire mass, and in this manner can again be made visible after it has almost disappeared through the action of water. In the one case the organic substance is confined to the nucleus; in the other it is pretty uniformly distributed through the entire mass of the sphæro-crystal. These sphærites rapidly disappear when acted upon by nitric acid. Concentrated sulphuric acid run in under the cover-glass turns them brown at once, and then quickly changes them into nests of crystals of gypsum (snlphate of lime), while the sphæro-crystals of inuline, on the other hand, appear unchanged.

If the dahlia tubers do not show sphæro-crystals of calcium phosphate, they can be readily found in other plants. They are certain to he found in the ground-tissue of the fleshy Euphorbias, cultivated in plant-houses, such as *E. Caput-Medusæ*, when this has been laid in spirit. They are found very beautifully in the pith of the inflorescence of *E. helioscopia* (the sun-spurge), when this is preserved in spirit.

Reactions of Tannin.—In order to demonstrate the tannin-reaction upon a typical object, we turn to the gall-apple or oak-gall, as found upon the leaves of our oaks. These gall-apples are due to the puncture of the oak-gall insect (*Cynips querci*), which lays an egg in the punctured tissue. We halve such a gall-apple while still young, and find on delicate *radial sections* taken from it that the interior hollow, occupied by the larva of the *Cynips*, is surrounded by a sheath, which consists of iso-diametric, rounded cells. These contain usually abundant

starch grains, turning blue with iodine. The tissue succeeding to this inner portion is formed of radially elongated, polygonal cells, which diminish in length at the periphery of the gall-apple, and finally end under the small-celled outermost layer, the epidermis, the cells of which are strongly thickened externally. This entire tissue, surrounding the inner sheath, shows no enclosures of definite form; it is traversed by vascular bundles which are visible here and there. If, however, we lay a freshly prepared section in a drop of watery solution of chloride or sulphate of iron, we see that it colours dark-blue throughout. This coloration is, moreover, communicated to the surrounding fluid, and gives us, therefore, the iron-blue reaction for tannin. If the action is observed under the microscope, by allowing iron solution to run into a dried section laid under a cover-glass, we see that first a fine dark-blue precipitate is formed, which, however, is soon again dissolved in the reagent, so that now a blue fluid fills the cells. The weakest tannin reaction is given by the starch-containing cells of the inner sheath.—For comparison, let us now lay a second section in a watery solution, about 10 per cent., of bichromate of potash, and we see a dense, flocculent, red-brown precipitate, which is permanent, formed in the tannin-containing cells. Or large pieces of tissue can be laid in potassium bichromate for at least twenty-four hours, and after washing them, sections prepared. The precipitate can then be accurately localised. Boiling potassium bichromate hastens the process.—Lastly, let us place a section in a concentrated solution of molybdate of ammonia in concentrated ammonium chloride, and an abundant reddish-brown precipitate appears in the cells. This reaction will decide in doubtful cases, because those preceding can also proceed from other reducing bodies.—Sections of dried gall-apples also give the above reactions, though less beautifully.

More roundabout, but specially certain, is the reaction with acetate of copper. The material to be investigated is, while living, cut up into small pieces, and placed in a saturated solution (7 per cent.) of acetate of copper, and allowed to lie therein for eight or ten days, or longer. Sections then taken are laid upon a slide in a drop of 0·5 per cent. solution of sulphate of iron. In this they remain for only a few minutes, as, after longer action, the walls commence to go brown. After the sections have been washed in water, and placed in a watch-glass of alcohol, in

order to remove the air and chlorophyll, they are examined in glycerine. The sections remain unchanged in glycerine or glycerine-jelly.—The pieces of plant can be removed from the acetate of copper into alcohol for preservation, and examined later, when desired, by the aid of acetate of iron. Iron-blue and iron-green tannins are clearly distinguishable by this method. If it is desired at the same time to fix the cell-contents, alcoholic solution of acetate of copper can be nsed instead.

Sclerenchyma Fibres of Vinca.—If a strong stem of *Vinca major* (the Periwinkle), cut off close above the ground, is broken, we see numerous small fibres project from the edge of the broken surface. We seize a tuft of such fibres with the forceps, draw them out, and place them in a drop of water on an object-slide. Under the microscope they appear as long, strongly thickened **sclerenchyma fibres**, tapering at both ends. The cavity is reduced to a narrow canal, which is entirely obliterated at both ends of the fibre, and sometimes at intermediate places. In less-thickened fibres the wall appears striate in one direction only; in more strongly-thickened fibres there are two opposite oblique systems of striæ, of which one belongs to the outer, the other to the inner system of layers of which the wall is composed. These are due to the spiral thickening of the wall.—With chlorzinc iodine solution the fibres quickly take on a violet colour, shading into brown. Specially instructive, however, is the relation with cuproxide ammonia, which reagent has the power of dissolving pure cellulose. The action must be observed direct. On the addition of the cuproxide ammonia solution the walls of the fibres swell strongly; at the first moment of the action the striation becomes more distinct, but quickly disappears. The outer complexes of layers are soon completely dissolved, while the inner one resists longer, and therefore the observer can see it completely isolated. At the beginning of the swelling a still finer stratification appears in the stratification which was previously visible; each layer is therefore composed of numerous exceedingly thin lamellæ. This fine stratification is stamped especially distinctly upon the inner more resistent complex.

Thickened Walls in Ornithogalum.—We now divide in halves, with the pocket-knife, a seed (not too old) of *Ornithogalum*, say *O. umbellatum* (the Star of Bethlehem), clamp the half in the hand-vice, damp the cut surface with water, and make with the

razor the thinnest possible section. This section (Fig. 26) shows us cells of approximately rectangular form. The walls of these cells are strongly thickened, the thickening layer being, however, pierced by numerous simple **pits**. If the section has skirted a cell-wall so that it offers a surface view, the pits appear as round pores (*m*), as can be seen in the upper cell of the adjoining figure. From the side the pits appear as canals, which extend from the cell-cavity up to the primary cell-wall. The pits of adjoining cells correspond, and are separated by the primary wall (*p*), which we shall designate the **closing membrane**. The inner surface of the thickening layer is distinguished by stronger refractiveness; it forms the **limiting layer**.—We now lay some sections in iodine, remove any superfluity, and run in a drop of dilute sulphuric acid. The thickening layers of the cells swell, and at the same time colour bright blue. Between these swelling thickening layers very delicate unswollen walls remain, and in thin parts of the section it can be determined that they have taken on a brownish tone. They are the so-called **middle lamellæ**[1] the walls which existed in the cells prior to the commencement of the thickening, and which also traversed the closing membrane of the pits.—In chlorzinc iodine solution the thickening layers of these cells colour dirty violet, and swell—the middle lamellæ again becoming brownish. In order to follow this reaction well it is desirable to put a dry section, without wetting in water, direct into the chlorzinc iodine. The cells are densely filled with protoplasm and granular reserve food-materials, the whole of which become yellow with chlorzinc iodine. In each cell the nucleus can be readily disclosed by the aid of acetic methyl green.

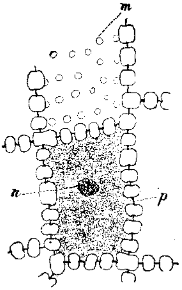


FIG. 26.—From the endosperm of *Ornithogalum umbellatum*. *m*, pits seen from above; *p*, closing membrane in pits seen in profile; *n*, nucleus (× 240).

As an alternative to this material, and offering very much the same characters, we may take the cortex of young twigs of the Cherry-laurel (*Cerasus Lauro-Cerasus*).

Thickened Walls in Date-stone.—The thickening layers of the cells in the endosperm of the seed or "stone" of the Date-palm

[1] See note on Rutheniumroth on page 433.

(*Phœnix dactylifera*) have a very similar appearance; the cells, however, are more elongated, their cavity narrower, the walls proportionally much thicker, and they are radially arranged. Cross and longitudinal sections, therefore, provided they correspond with the radii, show the cells in longitudinal view, while tangential sections, which cut the radii at right angles, show cross-sections of the cells. Here also iodine and sulphuric acid colour the thickening layers blue, chlorzinc iodine solution a very beautiful violet, and by slow swelling numerous lamellæ are usually brought into view.

"*Bordered Pits*" *of Pinus*.—We turn now to the pine-wood (*Pinus*, etc., any species, preferably *P. sylvestris*, the Scotch fir), in order to study **bordered pits.**[1] For this purpose we take a piece of wood, either dry, or, better still, preserved in alcohol, from a stem as old as possible. First we prepare with a sharp pocket-knife the suitable surfaces for cutting—one radial, parallel to the long axis of the stem, one tangential to the same, and one directed perpendicularly to this axis. The concentric yearly rings which are visible with the naked eye upon every piece of pine-wood will provide us with the necessary bases from which to decide as to the directions in question, and the *orientation* of the sections must be very carefully attended to. The *radial longitudinal section* cuts the yearly rings perpendicularly; the *tangential longitudinal section* is so much the more perfect, the more parallel it runs to the yearly rings. The *cross-section* is directed perpendicularly to both longitudinal sections.—In the following preparation of microscopical sections, in order that the sections shall be good, and not to damage the razor, quite special precautionary rules must be adopted. If the razor is hollow-ground, rightly directed sections can be taken only from the edges of the piece of wood, *i.e.*, so long as the back of the razor does not yet rest upon the cut surface. However, in general, only slightly hollowed razors should be used for cutting wood, as those greatly hollowed easily "give"; and indeed it is recommended to use razors which are ground flat on one side, *i.e.*, the side which will rest upon the cut surface, though these razors have the disadvantage that they are not easily sharpened. The cut surface must always be moistened; the sections must be as thin as possible. It is not necessary to have them of any size. A section which appears to be too thick should not be cut to the end; it is better to withdraw the razor from the cut in order not to notch the edge.

[1] See note on Rutheniumroth on page 433.

The razor must be as sharp as possible, otherwise it will tear the cell-walls, and separate the inner thickening layers from the outer. The wood preserved in alcohol cuts more easily than when dry, especially if the former has been laid for about twenty-four hours prior to use in a mixture of equal parts glycerine and alcohol. The surface which has been prepared by the pocket-knife, since it contains the torn cell-walls, must be removed with the razor in sections as thin as possible, and rejected. The succeeding sections can be used.[1]

A *radial longitudinal section*, rightly directed through the wood of the Pine, appears, with weak magnification, to be composed of longitudinally elongated cells, which overlap one another with their tapering, rounded ends. Running across these cells we see the cell-rows of the **medullary rays**, with which we shall not at present concern ourselves.—We focus now with a higher power upon a part in which we see only the walls of the longitudinally elongated **wood-cells**, always selecting the broader of them, and concentrate our attention upon the **bordered pits** of these walls. The bordered pit appears in the form of two concentric circles or ellipses (Fig. 27, *A*). The inner small circle, or ellipse, represents the opening of the pit into the cavity of the cell; the larger outer circle, or ellipse, the broadest part of the pit, with which it joins on to the primary wall separating the two elements. This pit, therefore, is only distinguished from the simple pit, as we have seen it in the Date and in *Ornithogalum*, in that it broadens at its base. The pits of the adjoining cells, however, coincide here in just the same way. If the mouth of

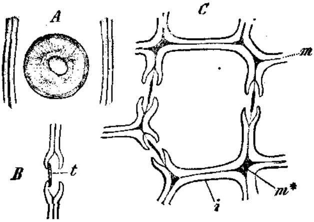


FIG. 27.—*Pinus sylvestris.* *A*, a bordered pit in surface view; *B*, a bordered pit in tangential longitudinal section; *t*, the torus. *C*, cross-section of an entire tracheide; *m*, middle lamella; *m**, a "seam"; *i*, the limiting membrane (× 540).

[1] It is of some advantage to keep an old thin razor (sharp, however), for preparing surfaces, as it is keener than a pocket-knife, and will spare the actual section razor. Even then the first section cut with the latter should be rejected.—[ED.]

the pit, as commonly is the case in the narrower cells, is an obliquely placed ellipse (as in *A*), by changing the focussing we shall find the corresponding mouth of the other pit oblique in the opposite direction. The two **pit-chambers** adjoining one another are separated from one another by the primary wall, which, before the commencement of the secondary thickening, was already present, and is only slightly thickened subsequently. This delicate wall is the **closing membrane.** In tho middle it is somewhat thickened, and forms the so-called **torus,** which is characteristic of the closing membrane of bordered pits. With most careful observation and suitable focussing with a sufficiently high power we may even be able to see this torus. It forms a round, weakly-shining disk, which has about twice the diameter of the mouth of the pit (compare in *A*). In the most favourable cases, and especially in preparations of dried wood, radial striation is observable in this torus, so that the delicate part of the closing membrane appears differentiated into radially arranged lamellæ (Fig. 27, *A*).

A complete insight into the structure of the bordered pit can only be obtained with the aid of *tangential sections.* As the bordered pits stand on the radial walls of the wood-cells, they are seen in cross view (Fig. 27, *B*) in correctly taken tangential longitudinal sections. We search for these structures in the walls separating the wood-cells, stopping first at the dividing walls of the broader wood-cells, and not allowing ourselves to be led astray by tbe sectional view of the medullary rays, which are formed of a number of smaller cells standing one over the other. The figure of the cut pit is, it is true, clear only in very delicate parts of the section. If this condition is fulfilled, the pit appears in the form of the two pairs of pincers directed towards one another (or like a couple of extremely short screws placed with their heads flat together), much as in the above figure (27, *C*). If once tbe structure of these large-bordered pits is known, we can easily realise the structure of the smaller ones, which lie in the thicker walls of the narrower wood-cells. The difference, apart from the smaller size, is, that here on both sides a longer canal, corresponding to the thickness of the wall, runs out of the broadened pit-chamber. Between the largest-bordered pits and the smallest are all intermediate stages. In the interior of the pit is seen, *in the most favourable cases*, the closing membrane, which in its centre is swollen into a **torus** (*t*). In the bordered

pits of the air dry wood it is usually pressed to one side of the pit-chamber (*B*). If, on the other hand, fresh wood, or alcohol material, is investigated, we shall find the closing membrane in the sap-wood (**alburnum**) stretched across the middle of the pit-chamber. In the heart-wood (**duramen**), on the contrary, the relations are just as we have given for the air-dry wood.

The figure of the bordered pit is clearer after the action of chlorzinc iodine, which stains the cell-wall yellow-brown. This coloration is due to the strong lignifaction of the walls. Here and there these walls appear bordered with violet on their inner sides. The closing membrane is not stained by the chlorzinc iodine. After treatment with this reagent we can readily convince ourselves that *the completely-developed wood-cells contain* neither protoplasmic sac nor nucleus; they consist only of dead cell-walls, and, as they serve for the conduction of water, and in this respect, as well also as in the nature of the thickening of their walls, resemble the tracheæ, *i.e.*, vessels, they are known as **tracheïdes.**

Not infrequently the thickening layers of the tracheïdes of the pine-wood, when examined in longitudinal section, show a more or less distinct spiral striation, mounting at an angle of about 50° to 60°. This arises from the formation of narrow spiral bands, instead of an uninterrupted thickening layer. The mouths of the pits then appear elongated in the direction of the striation, and like the striæ of the two adjoining side-walls, the mouths of adjoining pits cross one another.

We prepare also a *cross-section* of the pine-wood. This must be specially thin. The tracheïdes thus cut across appear as a rule rectangular. They form radial rows. The limits of the annual rings are marked out by the abrupt succession of the wide cavitied slightly thickened tracheïdes of spring, to the narrow cavitied greatly thickened tracheïdes of late summer. Each medullary ray is formed only of a row of narrow, radially elongated cells. *On the radial walls of the tracheïdes we see* the sections of the pits (Fig. 27, *C*), the figure of which appears in no way different from that in the tangential longitudinal section. Between the tracheïdes the **middle lamellæ** appear as fine separating lines (*m*). Where more than two tracheïdes are in contact, the middle lamella is thickened into a solid or hollow "seam" (*m**). The inner limit of the cell-wall surrounding the cavity is more strongly refractive, and forms the **limiting mem-**

brane (*i*), which is specially clear in the more strongly-thickened tracheïdes of late summer. It is still clearer after the action of concentrated sulphuric acid; the thickening sheaths swell, and are finally dissolved; the limiting membrane resists for a longer time and stands out sharply; between the swelling thickening layers are seen the primitive walls of the cells, of which finally only the delicate network of middle lamellæ remains, stained yellowish-brown. These middle lamellæ owe their resistance to concentrated sulphuric acid to a specially strong lignifaction which characterises the middle lamella of wood. Concentrated chromic acid solution acts upon a section in a similar way, and after prolonged action leaves of it only a network of middle lamellæ, appearing bright in the dark fluid. The resistance of this network indicates an especially strong lignifaction, which universally distinguishes the middle lamellæ of wood. With slower swelling in sulphuric acid, by usiug diluted acid, we can often determine, and especially in the strongly thickened tracheïdes of autumn, that the thickening layer consists of very numerous delicate lamellæ. With chlorzinc iodine the cross-section is coloured yellow-brown, the middle lamellæ a purer yellow; in individual cells, also, the inner thickening layer, immediately surrounding the limiting membrane, shows well the violet colour of cellulose; and if the section which has been in chlorzine iodine be also treated with dilute sulphuric acid (2 *parts acid 1 part water), in many cases the entire thickening* layer will take on a blue colour.

Exceptionally thin cross-sections of the wood of this Pinus serve as excellent **test-objects**, with the aid of which we can form a judgment upon the quality of the medium and higher magnification of our microscope. Sufficiently delicate sections being presupposed, the figure must appear perfectly flat, brightly lighted, sharp in outline, clear in structural details, and free from colour.

Reactions of Lignin.—In order to become acquainted with the characteristic reactions of lignified membranes (**lignin**) we will make use of phloroglucin and sulphate of aniline. We dissolve a trace of phloroglucin in alcohol, and lay some sections of wood in this solution. After this we place it in a drop of water on the object-slide, and allow, from under the edge of the cover-glass, hydrochloric acid to act upon it. The walls of the cells quickly take a beautiful violet-red colour. Other sections

we place in a watery solution of aniline sulphate, when they at once become bright yellow; this colour is still more heightened by the addition of dilute sulphuric acid. In place of phloroglucin we can use an extract, prepared with water or spirits of wine, from the wood of the Cherry, with almost the same result.—If sections of the *fresh* stem of the Pine, passing from cortex to pith, are treated with strong hydrochloric acid, a yellow coloration of the wood is at once produced, which, however, gradually gives place from the exterior inwards, and from the interior outwards, respectively, to a violet colour. This also is the phloroglucin reaction, and indeed proceeds from phloroglucin which is derived from the contents of the cortical cells and pith cells respectively. Even the medullary rays of the young wood contain a little phloroglucin, so that the violet colour also spreads from each of these. If the sections pass through the cambium we can readily see the gradual extinction of the lignin reaction in the neighbourhood of the cambium.

If cross-sections of pine-wood are laid in *Eau-de-Javelle*, and the action permitted for a suitable length of time, the lignin is removed from the lignified membranes, and the cellulose left behind will take the characteristic blue colour with chlorzinc iodine. Similarly the lignin can be removed by Schultze's maceration fluid.

CHAPTER VI.

THE EPIDERMIS—STOMATA—WATER-STOMATA.

Principal Materials Used.

Leaves of Flag Iris; fresh. Or, of Hyacinth.
Leaves of *Tradescantia virginica*, or *T. zebrina*; fresh.
Leaves of *Lilium*; in alcohol.
Leaves of *Aloë* or *Agave*; fresh; or alcohol material softened for twenty-four hours in half-and-half glycerine and alcohol.
Leaves of *Tropæolum*, or of *Fuchsia*; fresh.

Principal Reagents Used.

Chlorzinc iodine—Sulphuric acid—Tincture of alkanet—Potash.

Epidermis of Iris.—Take a surface-section of the outer side (morphologically the under side) of the "equitant" leaves of a Flag Iris, *Iris florentina*. The section must be so thin that it only grazes the tissue underlying the epidermis, and should be observed in water with the outer surface turned upwards. It will be seen at once that the **Epidermis** is composed of elongated cells which run parallel to the long axis of the leaf. The cells are ended by cross partition walls; they are connected together without any ordinary intercellular spaces, contain colourless cell-sap, a nucleus, and a very reduced protoplasmic sac. On its outer side the epidermis is covered by an exceedingly fine-grained layer of **wax**. In a line with the cells of the epidermis lie special intercellular spaces, the elliptic **Stomata**, which, however, are only indistinctly visible, because the four adjoining cells of the epidermis extend over the **Guard-cells** of the stoma,[1] partially

[1] In ordinary terminology, the term "stoma" includes the guard-cells and the accessory structures. I have limited the term, as etymologically it could be limited, to the pore ("stomatic cleft," of many authors). The [illegible]le structure is a "stomatic apparatus".—[Ed.]

covering them. Hence there remains only an elongated elliptical pit (*f*) which leads to the stoma (Fig. 28, *A*). This **stomatic pit** usually appears black, because filled with air.—In order to see the guard-cells well, now turn the section over. It can then be easily proved that the stoma is enclosed between two semilunar guard-cells. In distinction from the neighbouring epidermal cells these cells contain chlorophyll bodies. The nuclei usually show as clear spots about the mid-length of the guard-cells. Between the two guard-cells is a spindle-shaped cleft, the stoma proper or stomatic cleft (*s*), apparently about half the length of these cells.

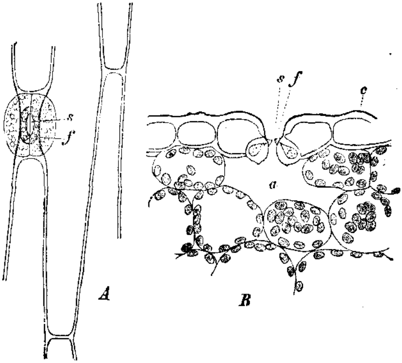


FIG. 28.—Epidermis of the under side of the leaf of *Iris florentina*. *A*, surface view; *B*, in cross section; *f*, stomatic pit; *s*, cleft, or stoma; *c*, cuticle; *a*, air-chamber (× 240).

Since the long axis of the stomata coincides in this instance with the long axis of the leaf, it is easy to obtain correct *cross-sections* of the stomata. The section should be taken at right angles to the long axis of the leaf. For this purpose a narrow strip, say about ½ inch long and $\frac{1}{10}$ inch broad, should be cut out of the leaf, in the direction of its length, with a pair of scissors; this strip can be supported between two pieces of the pith of the elder or of the sunflower, or, though less advantageously, between two pieces of carrot or of fine bottle cork; or, several such strips

can be packed together without other support than they give to each other. The elder or sunflower pith necessary for this purpose is obtained from dried pieces of the stems of those plants by stripping off the cortex and ring of woody tissue. A piece of pith about an inch long is cut in two lengthwise with a sharp razor. The flat strip of leaf which has to be cut is now laid between the two halves of the pith, so that the end of the strip reaches to the end of the split piece of pith. Thin cross-sections are then taken through pith and object at the same time, and the sections are lifted with a camel-hair brush from the blade of the razor on to the object-slide. While cutting, the two pieces of pith can either be held together simply with the fingers, or the two halves can be fastened together by winding round them a piece of thread. In cutting, the pith is so held that the razor passes through the strip of leaf from side to side and not from edge to edge; in this way many approximately equal sections can be taken. For delicate objects the softer sunflower pith is preferable to the somewhat harder elder pith; for more resistant objects, like that in question, elder pith is better used; for still more resistant objects, not pith, but fine cork, as used for bottles.

The preparation of sufficiently thin sections will probably offer no insuperable difficulties, but may be ensured by the use of a hand microtome, such as is illustrated in the annexed Fig. 29 (Leitz, Wetzlar, 15s.). The object to be cut is placed in the tube, fixed by the clamping screw, and adjusted level with the glass cutting table. The adjustment of the object before and after cutting is effected by means of the screw bottom, one division on the graduated tube of which indicates $\frac{1}{100}$ mm. ($\frac{1}{2500}$ inch). Cutting is performed by a razor, the blade of which is pressed flat on the glass cutting table. An ordinary razor can be used, or, better, one which is hollow on the upper, flat on its lower side.

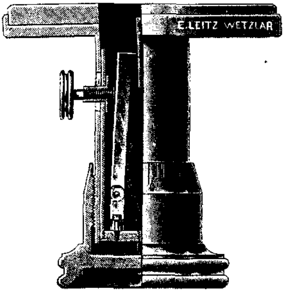


FIG. 29.—Leitz's hand microtome.

In this way a number of sections are prepared for further use, and they can be laid in the meantime, by means of a camel-hair brush, in a watch-glass filled with water. Place some of the sections in water under the microscope, and they will show, in favourable places, median cuts through the stomata, as shown in Fig. 28, *B*. As such a cross-section will show, the epidermal cells of *Iris florentina* are more strongly thickened on their outer than on their inner side. The inner walls, however, are also pretty thick, while the radial walls are only slightly thickened. This structure is connected with the function of the epidermis, which not only has to serve as an outer protecting sheath, but also has to serve as a water-reservoir. The thin radial walls easily allow a change in the capacity of the cells, which, by means of a bellows-like play, diminish in height with loss of water, and enlarge again with increase of water. The two guard-cells lie recessed between the epidermal cells; the manner in which the latter overlap the guard-cells can be at once seen. The stomatic-pit leads down to the guard-cells. These latter are strongly thickened on the upper and under side. Above the cleft is found a special thickening ridge which in cross-section constitutes a beak-like projection. Under it the wall is for a short distance thinner, and the same over a broader area on the other side, towards the adjoining epidermal cell. This thickening of the wall is connected with the mechanism of movement of the guard-cells, which would become higher and curve more strongly, thus widening the cleft, when their turgidity increases; but their curvature would diminish, they would flatten, and thus narrow the cleft, when their turgidity decreases. In order that the movement of the guard-cells may not be hindered, the strongly-thickened wall of the surrounding epidermal cells thins suddenly at their line of union with the guard-cells; the guard-cells are here attached as with hinges—the stomatic hinges. Under the stoma is found an air-cavity, the **ventilating chamber** (*a*), a large intercellular space, under natural conditions filled with air, surrounded by chlorophyll-containing cells, and connected with the intercellular spaces which are found between them.—A *cross-section* laid in chlorzinc iodine shows us that the walls of the epidermal cells stain violet in their entire extent, with the exception of a thin outer layer, somewhat wrinkled, the so-called **Cuticle** (*c*), which becomes yellowish-brown. This cuticle swells out at the stoma into the beak-like projection which we have already men-

tioned, which appears coloured yellow-brown by the chlorzinc iodine, *because it is cuticularised.* As an *extremely delicate* membrane, the cuticle is continued through the stomatic cleft, over the guard-cells, to the commencement of the chlorophyll-containing parenchyma. Apart from this, the guard-cells are also violet over their whole wall. By the use of concentrated sulphuric acid the whole section is dissolved, the cuticle alone remaining behind, together with the cuticularised projections of the guard-cells.

Stomata of Hyacinth.—The stomata on the leaves of various species of Hyacinth are constructed exactly as in *Iris,* though not sunk in the epidermis (*i.e.*, there is no stomatic pit), the *two guard-cells lying at approximately the same level with the* surrounding epidermal cells. Surface-sections, therefore, or *epidermal strips torn off, show the two guard-cells when viewed* right way up. The stomata are found not only on the under, but also on the upper, side of the leaf. As the long axis of the stoma in this case also coincides with the long axis of the leaf, it is easy to obtain cross-sections. At half-way up on the side towards the cleft, as well as on the whole side facing the surrounding epidermal cells, the wall of the guard-cells is slightly thickened. The structure of the "hinge" is the same as in Iris.

Stomata of Tradescantia.—An exceedingly favourable object for the study of the stomatic apparatus is found in the hardy spider-wort, *Tradescantia virginica.* The epidermis on both sides of the leaf consists of polygonal cells, mostly elongated in the direction of the long axis of the leaf; with these alternate narrow stripes of longer and *narrower cells.* These stripes are visible with the naked eye, especially on the under surface of the leaf, and appear green in colour, while the stripes of broader cells appear grey. The lateral walls of the epidermal cells are pitted; the outer surface is faintly striate. The number of stomata is markedly greater on the under side of the leaf; therefore we choose this side for examination. The stomata are almost always surrounded by four epidermal cells (Fig. 30, *A*). The guard-cells lie on the same level with the epidermal cells; the cleft which they have between them is comparatively large; they contain chlorophyll grains, between which the nucleus is usually visible. In the *epidermal cells also the nuclei are very* conspicuous, and appear surrounded by small colourless leucoplasts (Fig. 30, *A*, *l*); the cell-sap of the epidermal cells is here and

there rose-coloured.—The long axis of the stoma corresponds with the long axis of the leaf, so that here also it is easy to obtain correct *cross-sections*. The stomata present then the appearance shown in Fig. 30, *B*. The side of the guard-cells towards the cleft appears in this case also to be less thickened at about mid height, while the whole of each side turned towards the adjoining epidermal cells is also thinner. Besides this, it happens that both of the epidermal cells adjoining the guard-cells are flatter than the epidermal cells lying beyond, and are also less thickened on their outer sides. They appertain, therefore, to the stomatic apparatus as "accessory cells," and stand in lieu of the hinge or joint which in *Iris florentina* is formed merely by the thin part of the membrane at the junction with

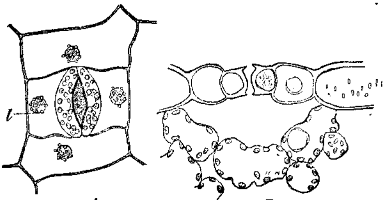


FIG. 30.—Epidermis of the under side of the leaf of *Tradescantia virginica*. *A*, seen from above; *B*, in cross-section through the leaf; *l*, leucoplasts upon the nucleus (× 240).

the guard-cells. The leucoplasts (*l*), which surround the nucleus *in the epidermal cells offer here a very favourable object for* observation. It is interesting that these leucoplasts, in spite of being in a position so strongly exposed to the light, remain small and colourless, and do not develop into chlorophyll grains; but this is connected with the fact that the epidermis has here no assimilating function.

Epidermis of T. zebrina.—Tradescantia zebrina has a stomatic apparatus composed in the same way, and can replace *T. virginica* when the latter is not available. Stomata are present only on the under side of the leaf. Many epidermal cells contain red cell-sap. On each side of the stomatic apparatus not one but two accessory guard-cells are usually present. The *cross-section* is very instructive, though not easy to obtain sufficiently thin;

thicker sections, however, serve for general information. The epidermal cells on both sides of the leaf are distinguished, as cross-sections show, by their considerable height; those on the upper side especially are so high that they alone form half the thickness of the leaf. Many of these epidermal cells are seen to be divided by cross walls. The leaves of *Tradescantia* possess, therefore, in their epidermis, a specially efficient water-reservoir. The accessory cells of the stomata are, as the cross-section shows, quite flat and thin, so that a great air-chamber, of the depth of the surrounding epidermal cells, is formed under the stomatic apparatus.

Stomata of Lilium.—Very beautiful large stomatic apparatus are present on the under side, more rarely the upper side, of the leaves of the white garden lily, *Lilium candidum*, and this can therefore be recommended as an object for investigation. The epidermal cells are elongated in the long axis of the leaf, lie in straight rows, but have, however, an undulating outline. The stomatic apparatus stand as prolongations of the epidermal cells, and at the same height with them. The cross-section is easy to obtain, and shows a hinge at the point of junction of the guard-cells in the form of a sudden thinning of the strongly thickened outer wall of the neighbouring epidermal cells.

Epidermis of Aloë.—The species of *Aloë* and *Agave* possess epidermal cells thickened very strongly on their outer sides, and stomata proportionally deeply sunk in the epidermis. Because it is specially instructive, and not difficult to prepare, we select for observation *Aloë nigricans*, a greenhouse plant with ligulate leaves arranged in two series (ranks); but other species of *Aloë* can serve as substitutes. In *surface-sections*, the epidermis of upper as well as under side appears formed of regular polygonal cells, mostly hexagonal. The cavity (or lumen) of each of these cells is reduced to a relatively small, rounded space, which appears dark, because in cutting it has become filled with air. The stomata are found on both sides of the leaf; deep pits lead up to them. These stomatic pits are always bounded by four cells, and have a rectangular contour; a somewhat prominent rim surrounds the pit. If you wish to see the guard-cells, it suffices to lay the sections on the glass-slide with their inner side upwards. The guard-cells are comparatively broad and short; amongst their contents are noticeable strongly refractive spherical oil-globules.—As the epidermis is very hard, the *cross-section* is best taken between two pieces of bottle cork. The

whole thickness of the leaf need not be taken, but a piece of the tissue, say about $\frac{1}{25}$ inch thick, is cut off from one surface of the leaf. As the stomata run parallel to the long axis of the leaf, we arrange the piece of leaf so that it shall be cut at right angles to this axis. We cut the sections from the inner towards the outer, *i.e.*, from the soft towards the harder part of the tissue. The stroug thickening of the epidermal cells is at once noticeable in these *sections (see* Fig. 31); this thickening affects only the outer half of the cell; corresponding to it, the cavity of the cell narrows outwards. The thickened parts of the cell-wall are white, strongly refractive, and are covered externally by *a cuticle more* strongly refractive still, but not sharply delimited. The lateral boundaries of the cells are only indicated by delicate lines in the thickened mass, and externally by a slight ridge. The interior of the strongly refractive thickening layer is covered by a comparatively thin, feebly refractive layer (*i*). This surrounds especially the keel-shaped narrowed part of the cell-cavity; while gradually thinning off, it ends on the side walls simultaneously with the refractive thickening layer. This thickened part of the epidermis, viewed in the aggregate in the section, appears like a curtain cut into regular scollops. At the places where a pit leading up to a stoma is found, is first to be noticed the projection which encloses the stomatic pit as with a rim; next, that the scollop, formed by the thickening layers, is here only half as wide, and has also only half its usual depth. The guard-cells show, both above and below, on the stomatic side, projecting ridges (of "entrance" and of "exit"), which in cross-section appear beak-like. Above the guard-cells are found the thin parts of the wall which serve as epidermal hinges. The ventilating chamber is narrow and deep.—Commonly a parallel, more or less oblique, striation will be observed on the thickened

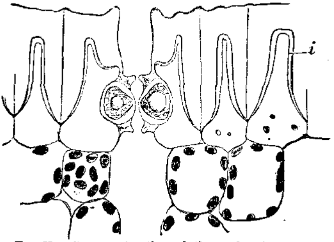


FIG. 31.—Cross-section through the epidermis and stoma of *Aloë nigricans*. *i*, inner thickening layer (× 240).

walls of the epidermal cells; it is caused by the razor in cutting, and not infrequently recurs with hard elastic objects.

A section treated with chlorzinc iodine, shows the highly refractive thickening layer coloured yellow-brown; it is, therefore, cuticularised. The inner covering to this layer (*i*) is, on the other hand, coloured violet, as likewise are the other cell-walls of the leaf. The yellow-brown coloration passes over the "hinge" on to the outer and inner ridges which are on the guard-cells. Elsewhere the guard-cells are coloured violet.—On treatment with concentrated sulphnric acid, the whole of the part which colours yellow-brown with chlorzinc iodine remains undissolved for a time, bnt after some hours' action this also is dissolved, and then the delicate cuticle, and the fine middle lamellæ found between the epidermal cells, alone still persist. The cuticle is continued over the guard-cells to the junction with the chlorophyll-containing inner cells. The cuticular layers and the cuticle take a brown colour in the sulphuric acid. The oil present in the guard-cells "balls" together, immediately on the entrance of the acid, into a highly refractive spherule, which disappears after some time.—In sections laid for a few hours in a solution of alkanet in 50 per cent. alcohol the cuticle becomes red; any fats present take a deeper red colour, so that this coloration of the cuticle is taken to show the presence of fatty substances in it.

Water-Pores of Tropæolum.—We will now turn our attention to **Water-Pores** or **Water-Stomata.** These show the same structure as the air-stomata, but are larger, and the cleft, as well as the underlying intercellular space, is, at least in part, filled with water. The guard-cells of these stomata as a rule quickly die, and then lose their movability, so that the cleft between stands wide open. The most favourable object for the study of these water-pores is *Tropæolum majus* (the Indian cress or so-called "Nasturtium").

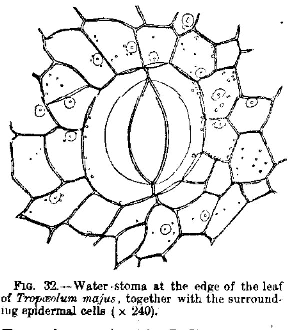


FIG. 32.—Water-stoma at the edge of the leaf of *Tropæolum majus*, together with the surrounding epidermal cells (× 240).

The water-stomata are found in the upper side of the leaf, and always over the ends of the principal veins. Here the edge of the leaf tends to show a small depression. A general idea of the water-stomata can be obtained if a suitable piece of a leaf in its entire thickness is brought into the field of the microscope, under water, and covered with a cover-glass, though the details are only observable on surface-sections taken from a suitable part of the edge of the leaf. A water-stoma then presents the appearance in Fig. 32. The dead contents of the guard-cells are as a rule very greatly reduced. Several water-stomata are usually found grouped at a short distance from one another.

Water-Pores of Fuchsia.—Another favourable object for the study of these water-pores is the *Fuchsia*, but preparation is somewhat more difficult, as the stomata are only found at the apex of the teeth on the leaf margin. The sections must therefore be taken through these. The guard-cells here show living contents, and a fair number of chlorophyll bodies. Thick sections can be made transparent with potash.

CHAPTER VII.

THE EPIDERMIS (*Contd.*)—HAIRS—GLANDS—WAX.

Principal Materials Used.

Leaves of Wallflower; fresh. Also young shoots; in alcohol.
Leaves of *Matthiola annua* (Ten-week Stock); fresh.
Flowers of Pansy; fresh.
Flowers of *Verbascum nigrum* (Mullein); fresh; also,
Leaves of *V. thapsiforme*, or *V. thapsus*; fresh.
Leaves of *Shepherdia canadensis*, or of *Elæagnus angustifolia*; fresh.
Twigs of *Rosa*; fresh.
Young stems and leaves of Stinging-nettle (*Urtica dioica*); fresh.
Petioles of *Primula sinensis*; fresh; or of *Pelargonium zonale*.
Young shoots of Pansy (*Viola tricolor*); fresh.
Winter buds of Horse-chestnut (*Æsculus Hippocastanum*); fresh, and in alcohol.
Leaves of *Echeveria*, and *Eucalyptus globulus*; fresh.
Nodes of stems of Sugar-cane (*Saccharum*); fresh.

Principal Reagents Used.

Acetic acid—Sulphuric acid—Hydrochloric acid—20 per cent. chromic acid —Rosaniline violet.

We are already acquainted with the **root-hairs** of the Frogbit, *Hydrocharis morsus-ranæ*, and as root-hairs are always, like these, unicellular sacs, we need not further investigate them. We have also seen the epidermal cells of numerous petals elongated into conical **papillæ** (*Tropæolum*, *Rosa*), and also the staminal hairs of *Tradescantia*, threads formed of barrel-shaped, swollen cells (Fig. 14); lastly also the hairs of *Cucurbita*, simple pointed threads arising from a multicellular base. Plant hairs of various forms are therefore already known to us; it is, however, worth while to amplify our special knowledge of them.

Hairs of Wall-flower.—On the leaves and stems of **Cruciferæ** we find very many forms of much-branched **unicellular hairs**. On

the stems and leaves of the Wall-flower, or Gilly-flower (*Cheiranthus Cheiri*), we see spindle-shaped structures (Fig. 33, *A*), with narrow cavities obliterated towards the two ends. These unicellular spindles are covered on their outer surface with protuberances, less numerous larger ones and more numerous smaller ones between them. As the spindles are all directed parallel to the long axis of the leaf, we can see in what direction *cross-sections* of these should be taken, though thin sections are not easy to obtain. As it is desirable to hit upon the hair at its point of insertion in the centre of its length, numerous sections should be taken in order to increase the chance of success.[1] We see (Fig. 33, *B*) that the place of insertion of the hair lies somewhat sunk, and that the epidermal cell which broadens out into the body of the hair is narrower than its neighbours, that at the base it is somewhat swollen, rounded, and extends farther into the surrounding tissue. It forms the "foot" of the hair. Longitudinal sections through the leaf show that the foot is not broader in the long direction of the hair than in the cross direction. We can readily satisfy ourselves that the cavity of the foot passes without interruption into the cavity of the body of the hair.—We can obtain a still more complete idea of the form of the foot if we lay a thin *surface-section* with the under side upwards. The foot is circular in section. It can now be seen,

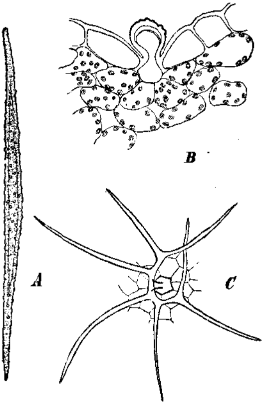


FIG. 33, *A* and *B*.—From the under side of the leaf of *Cheiranthus Cheiri*. *A*, the hair seen from above (× 90); *B*, in cross-section (× 240); *C*, from the under side of the leaf of *Matthiola annua*; hair seen from above (× 90).

[1] As similar hairs are found longitudinally placed upon the young stems, and these latter are much easier to prepare cross-sections of, they may offer alternative material, and if preserved in alcohol are so much the better.—[ED.]

also, that the chlorophyll-containing cells of the tissue of the leaf join radially, and without gaps, with the somewhat broadened part of the foot projecting below the epidermis.

Hairs of Matthiola.—The hairs of the ten-week stock, *Matthiola annua* (Fig. 33, *C*), are repeatedly branched in one plane parallel to the leaf surface. These hairs, especially on the under surface of the leaf, are set so closely together that their branches interlace. The cavity of the hair, in consequence of the strong thickening of the walls, is well-nigh obliterated. Knobs are scarcely at all developed on the surface.—The view of the epidermis from the inner side (by means of *surface-sections* placed upside down) is very instructive, for it shows a tolerably marked swelling of the globular foot of the hair, and around it an exceedingly beautiful radial arrangement of the chlorophyll-containing cells.

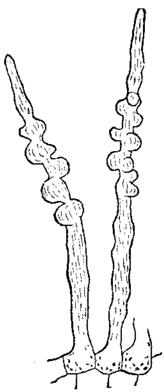


FIG. 34.—Hairs from the furrow of the lower petal of *Viola tricolor* (× 240).

Hairs of Pansy.—In the groove which leads to the spur-like elongation of the lower or anterior petal of the pansy (*Viola tricolor*) are very peculiar long unicellular hairs (Fig. 34). They can be seen very well if a *cross-section* of the lower petal is taken near the place where the tubular spur opens out into the furrow or groove. Each of certain epidermal cells grows out, almost in its entire width, into a hair. This is covered with irregular gnarled swellings. The cuticle of the hair shows slight longitudinal ridges. The cell-sap is colourless, but yellow pigment-bodies (chromatophores) are often present in the protoplasmic sac.

Staminal Hairs of Mullein.—The staminal filaments in the flowers of the common Mullein (*Verbascum nigrum*) are covered with unicellular violet hairs. In order to examine them the anther should be removed from the filament, and this latter pulled to pieces with needles in a drop of water on an object-slide. The hairs are very long, swollen out at the end into the form of a club, and with violet cell-sap. The surface of the hair is covered with somewhat elongated protuberances which ascend in more or less regular spirals.

Branched multicellular hairs are to be found in the same plant on the under side and edges of the corolla. Seen from above, these hairs have a certain likeness to those of *Matthiola*, but all of the branches here arise from a common centrla point, and each branch is in itself a closed cell. Moreover, the branches do not spread out in the same plane, but arise at indefinite angles. Their walls are quite as strongly thickened as in *Matthiola;* outer protuberances are wanting. The hairs on the edges of the petals are seen in side view. The body of the hair is cut off by a partition wall from the epidermal cell which bears it. It consists of a stalk or pedicel, almost always unicellular, and upon this the branches are mounted. Slight modifications of these conditions occur, which need no further explanation.—Besides these branched hairs, the edge of the corolla also bears small glandular hairs. These have a two to three celled stalk, and a flattened head, which is covered here and there at the apex by a strongly refractive substance. These last we shall not, however, study here, but in another more favourable object.

Felted Hairs of Mullein.—It is only necessary to imagine the multicellular branched hairs of the Mullein placed one upon another several times, in order to understand the hairs which form the felt on the leaves of *Verbascum thapsiforme.* These hairs are sometimes as many as five stages high, each stage separated from its predecessor by a unicellular joint, which continues the main axis of the hair. The cells of the hair are for the most part filled with air. They are best shown by cross-sections through the midrib of the leaf.[1]

Scales of Shepherdia.—To the same category as the branched hairs of the petals of *Verbascum* belong the scales of *Shepherdia canadensis*, a hardy ornamental Elæagnaceous shrub. On the under side of the leaf, distinguishable even with a hand lens, we find more or less loosely constructed white, and more or less closely constructed brown (Fig. 35, *A*) stars. On the upper side of the leaf only white stars are to be found, and always in smaller number. The cells of these looser white stars contain, as microscopical examination shows, only air; they arise from a common central point, but are separated from one another laterally. On the upper side of the leaf they do not lie in one plane,

[1] *Verbascum Thapsus*, a native perennial, has very similar hairs.—[ED.]

but rather radiate stellately in all directions. The cells of the brown stars are connected together almost to the tip, and provided with living contents; the nuclei in their interior can be seen without difficulty. A *cross-section* through the leaf, where it cuts a brown star centrally, shows that its stalk (Fig. 35, *B*) is multicellular, and that not only the epidermis but also the cell-layer next beneath passes over into it.[1] The stalk bears aloft the stellate unilamellar but multicellular expansion.

Should *Shepherdia canadensis* not be at our disposal, *Elæagnus angustifolia* can to a certain extent replace it. Here, on the under side of the leaf, only the white air-containing scales are present. The disk consists of cells either laterally isolated, or grown together almost to the margin.

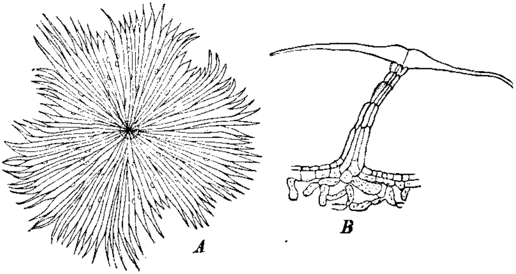


FIG. 35.—Scales from the under side of the leaf of *Shepherdia canadensis*. *A*, from the surface; *B*, in cross-section (× 240).

Prickles of Rosa.—Now take a horizontal section through the stem of a rose, say *Rosa semperflorens* of gardens, at the place where one of the prickles arises. Try to halve the prickle as nearly as possible in the middle, and then to take a thin section,—a process not so easy as it seems. In cutting, do not neglect to moisten the cut surface with water. In a successful section it can be seen that the epidermis of the stem is continued over the prickle, the cells being at the same time more strongly thickened and more elongated. Inside the epidermis there pass into the

[1] This (as also *Rosa*, following) should preferably be classed as an emergence, rather than a hair proper, since it includes also tissue of subepidermal origin.—[ED.]

prickle pretty strongly thickened narrow-cavitied cells, and, farther in, similar but broader ones. These last fill up the whole central part of the prickle. All these cells are finely pitted. The epidermis of the stem is separated from the chlorophyll-containing inner tissue by an often strong layer of considerably thickened elongated cells joining on to one another with oblique end-walls, and containing no chlorophyll. These cells without chlorophyll are of like origin to those which form the inner tissue of the prickle. The tissue-elements of the prickle are, however, separated from the chlorophyll-containing tissue of the stem by a layer of flat-celled tissue, which arises by division from the undermost layer of the tissue of the prickle; it follows only for a short space the chlorophyll-containing tissue of the stem, and then turns towards the epidermis, in order to limit the base of the prickle laterally also towards the chlorophyll-less tissue of the stem. This is a **cork-layer**, next to the outer surface of which, by the intercalation of a layer of separation (**absciss-layer**) the fall of the prickles will result in the older parts of the stem. Even before this, it is possible to break off the prickle pretty smoothly from the stem, along the surface of the cork-layer.—*If we select a prickle from the leaf-stalk* for examination, its structure is found to be in no way different from that on the stem, excepting that at its base the cork-layer is wanting. Since the leaf as a whole will fall, separate provision for the fall of the prickles is unnecessary.

By careful examination of the cortical tissue adjoining the prickles of the rose, the presence of **crystals** in the cells can be made out. As they are not dissolved in acetic acid, nor in potash, but on the other hand are dissolved in hydrochloric or sulphuric acid without evolution of gas, they are crystals of oxalate of lime (see p. 63). They have here the form either of monoclinic prisms or of **cluster-crystals.** These last consist of a great number of partial crystals which are deposited on an original crystal. The cluster-crystals are conspicuous from their size and stellate form.

Stinging Hairs of the Nettle.—In order to get the **stinging hairs** of the common stinging nettle (*Urtica dioica*) uninjured, we must take them from the younger parts of the plant. They are found best on the veins of young actively growing leaves. The hair, which is visible with the naked eye, should be cut off below its point of insertion with the razor, and examined in

water. If the hair is already dead, air will be found in its interior, and its apex is then no longer intact. An uninjured hair presents the appearance represented in Fig. 36. The hair is unicellular, sharply tapering, swollen at its apex into a small knob. At the base the hair broadens out, and the bulb thus formed is sunk in a cup which is developed from the tissue of the leaf. As its developmental history (ontogeny) shows, this hair springs from a single epidermal cell, lying at the same level with its neighbours; afterwards the strongly swelling foot of the hair is lifted up on a column of tissue, which is covered by the epidermis, and is formed internally of hypodermal (sub-epidermal) tissue.[1] In the hair itself is to be seen streaming of the protoplasm. The nucleus is usually to be seen within the bulb, suspended by protoplasmic threads. The cuticle shows oblique striæ, which ascend in the same direction in all the hairs. The wall of the terminal knob, and the parts of the hair in its neighbourhood, are silicified through their entire thickness. This can be proved by treatment with concentrated sulphuric acid, and subsequent addition of 20 per cent. chromic acid, by which means the organic membrane is completely removed and a siliceous skeleton left. Lower down the thickness of the silicified portion rapidly diminishes, and ultimately is limited to the cuticle. As the ebullition upon treatment of the hair with hydrochloric acid shows, the unsilicified portions of the wall are, on the other hand, impregnated with carbonate of lime, by which the rigidity of the hair as a whole is enhanced. As already noted, hairs are often found with their points broken off. The breaking-off of the knob is facilitated by the wall of the hair just under it having a thin part. The oblique insertion of the knob, on the other hand,

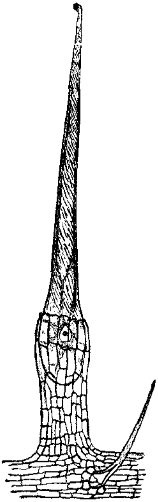


FIG. 36.—Stinging hair of *Urtica dioica*, together with a fragment of the epidermis, on which is a small bristle (× 60).

[1] That is, the hair is borne upon an emergence.—[ED.]

results in the broken surface being sloped downwards, and thus a sharp point to the hair is produced. It will also be seen that though we speak of the hair as ending in a knob, this latter is finer than the finest needle, and hence, in case of careless handling, the hair pierces the skin, and being very brittle, the knob breaks off, and the strongly acid sap enters the wound and causes slight inflammation.—On the same piece of the epidermis, near to the stinging hairs, are also small unicellular bristles (*cf.* Fig. 36), which are distinguished by the strong thickening of their walls, and their fine tapering points.—Similar bristles are found on the edge of the leaf. For this purpose it suffices to place a piece of the leaf in water uuder a cover-glass. In old leaves the bristles can be thickened almost to the obliteration of their cavity; their surface is covered with small protuberances.

Glandular Hairs in Primula.—We bave already found **glandular hairs** on the edge of the petals of *Verbascum nigrum;* they can be studied under more favourable conditions in the common greenhouse *Primula, P. sinensis.* For this purpose *cross-sections* are taken through a leaf-stalk. The body of the hair is divided from the epidermoid foot-cell by a cross wall situated out beyond the epidermis, and forms a cell-row, which consists of usually two (sometimes more), longer and broader, and one (rarely two), narrower and shorter cells. The last cell bears the glohular **head**, and upon this is borne a more or less strongly-developed cap of highly refractive resinous yellowish substance.—The secretion of this takes place between the cuticle and the rest of the cell-membrane. The cuticle is raised, distended, and finally ruptured, whereupon the secretion overflows the upper part of the hair. The addition of alcohol removes the secretion, and then the raised cuticle can he clearly seen lying in folds. The cells of the hair show a beautiful network of protoplasm, with suspended nucleus, in which lies a large nucleolus. Small chlorophyll bodies are embedded in the peripheral protoplasm.

Hairs of Pelargonium zonale.—Instead of *Primula sinensis*, and, like it, available all the year round, we can use the ordinary zonal bedding "geranium," *Pelargonium zonale*, in any of its innumerable varieties. The glandular hairs have exactly the same structure as those of *Primula*, and can be very conveniently studied upon *cross-sections* through a full-grown but not too old petiole. This plant is indeed a very favourable object for the study of hairs generally, for they are present in various typical

forms, with transitions. Two distinct types can be recognised, **conical** and **capitate.** The conical hairs are either (1) delicate and unicellular, (2) stouter, and with one or two partitions, the septum, where solitary, being commonly quite close to the outer line of the epidermis, or (3) very long and tapering, with many cross septa, and a distinctly bulbous base, raised upon a wart-like outgrowth, the cells of which show radial arrangement. The capitate hairs are (1) glandular hairs with a round head-cell on a short stalk, 2-3 cells long, (2) glandular hairs with an obovate head-cell set somewhat obliquely on a short stalk; (3) doubtfully glandular with a large pear-shaped head-cell on a long 2-3 celled stalk. Between these forms various intermediate states exist. These hairs may also be studied in surface-sections; and in both these and in cross-sections, carefully taken from fresh material, the tapering or conical hairs not infrequently show **streaming movements** of the protoplasm.

Stipular Glands of the Pansy.—The large stipules of the pansy, *Viola tricolor*, are very deeply toothed, and bear at the apex of each tooth a beautiful egg-shaped gland. If it is desired to see these not shrivelled, but as in active life, it is necessary to investigate the youngest possible stipules. The gland (Fig. 37) is separated from the edge of the leaf by a somewhat narrowed neck. It consists of two or more rows of elongated cells, forming a core, upon which a single layer of cells is placed, arranged perpendicularly to the surface, and elongated in this direction. The figure shows such a glandular hair in optical longitudinal section. The whole gland is distinguished by its copious protoplasmic contents. In these are often to be seen vacuoles filled with cell-sap, either singly or in groups. The secretion consists of a thin layer of resin on the surface of the gland, and of masses of slime, which raise the cuticle. With rosaniline violet the contents of the cells are coloured red, the resin layer blue, the slime masses reddish.

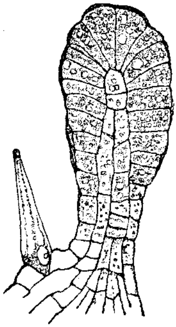


FIG. 37.—Glandular hair from the stipule of *Viola tricolor*, and an unicellular hair close by (× 240).

Glands on the Winter Bud of Æsculus Hippocastanum.—A

cross-section through a winter bud of the horse-chestnut (*Æsculus Hippocastanum*) shows us button-shaped glandular hairs, situated on the protective scales of the bud (Fig. 38). The intermediate scales of the bud bear glands on both sides; on the external ones more are found on the inner side, on the inner scales the most on the outer surface. The structure of the glands in *longitudinal section* is shown in the figure; there is an axial cell-row, which towards the top divides, and from which the secreting cells radiate. The cuticle is ruptured by the secretion, and this is discharged between the scales, coating them and sticking them together. This secretion consists of a mixture of **gum** and **resin.** In water the gum-drops scattered in the resin can be seen to swell; while, on the other hand, by the addition of rosaniline violet the resin mass is coloured a beautiful blue, and the contents of the glands are red.—The winter buds of the horse-chestnut can also be preserved as alcohol material. Section-cutting from this material is easier, and the glands can be more readily studied; but, naturally, the resin will have been removed by the alcohol.

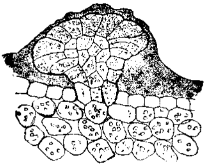


FIG. 38.—Glandular hair on a scale of the winter bud of *Æsculus Hippocastanum* covered with secretion (× 240).

Wax.—On one object (*Iris florentina*) we have already drawn attention to the finely granular layer of **wax** which covers the outer surface of the epidermis; we propose, however, to specially study it upon some other plants.

Very suitable for this is *Echeveria secunda-glauca*, or other similar plant, so often used in gardens for "carpet-bedding". The wax-layer, which can easily be wiped off, gives to the plant a hoary or "glaucous" appearance. A surface view of the epidermis shows us a net-like crust of aggregated grains.

Aggregated short rods forming a wax-layer can be seen in an easily observed form in the surface view of the epidermis of *Eucalyptus globulus*, the Australian blue-gum tree.

Similar delicate rodlets are shown upon cross-sections of the surface of adult internodes of the Rye, *Secale cereale.*

The most beautiful object, however, is the sugar-cane (*Saccharum officinarum*), so commonly cultivated in the plant-houses of botanical gardens, etc. Here the wax covering appears in the

form of long rods or filaments, often curved or curled at the end. We remove a surface-section from the nodes of the stem, which are noticeable from their glaucous appearance. As much air clings between the rods, it is best to immerse the section for a short time in cold alcohol; it can be then readily examined. On the

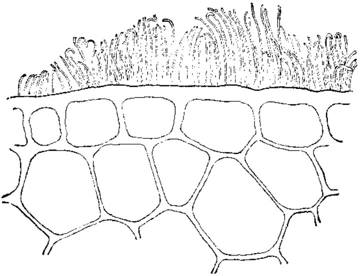


FIG. 39.—Cross-section through a node of the stem of *Saccharum officinarum*, with a rod-like wax layer (× 540).

other hand, it is difficult to obtain a good cross-section with the rods still adhering. Fig. 39 shows such an one. The rods stand closely crowded together, many showing the flexure already referred to. If a surface-section is brought near a flame, the rods, when examined under the microscope, are seen to be fused together. They dissolve in hot alcohol.

CHAPTER VIII.

THE MONOCOTYLEDONOUS STEM, AND CLOSED COLLATERAL VASCULAR BUNDLES.

PRINCIPAL MATERIALS USED.

Stems of *Zea Mais* (Maize, or Indian Corn); in alcohol. Or, stems of Oat, or other grass.

Full-grown leaf of *Iris florentina*, or the Flag Iris, in alcohol, and laid for twenty-four hours in half-and-half alcohol and glycerine. Also fresh material.

Stems of *Dracæna* (*Cordyline*) *rubra;* fresh; also in alcohol.

PRINCIPAL REAGENTS USED.

Chlorzinc iodine—Soda corallin—Safranin—Various stains for single or double staining—Borax-carmine and methyl-green—Hydrochloric acid.

Stem of Zea Mais.—A very favourable object for the study of the structure of the **closed collateral vascular bundles** of Monocotyledons is the stem of the Maize or Indian Corn (*Zea Mais*). We will examine material which has lain for some time in alcohol, in order the more readily to study the cell-contents at the same time. First prepare a *cross-section*, taking care that it passes through an internode and not through a node. We shall understand the structure more easily if the section is laid at once into a drop of chlorzinc iodine. Coloration of the section begins at once, and the separate **vascular bundles** stand out quite clearly even to the naked eye. If we lay the glass-slide on a white background (*e.g.*, a sheet of paper), we can in the simplest possible way see the **isolated** (or scattered) arrangement of the vascular bundles; an arrangement, as a whole, peculiar to Monocotyledons. It will also show that the vascular bundles are more closely crowded together towards the periphery of the stem. Every vascular bundle shows in cross-section as an oval spot; the tissue in which these bundles are embedded

is the **Ground** or **Fundamental Tissue.** A clear separation of the ground-tissue into pith and cortex is not generally present with the scattered or isolated arrangement of the bundles. Nevertheless, the whole of the internal tissue in which the vascular bundles lie, and including these bundles themselves, can theoretically be distinguished from the enveloping primary cortex under the name of the **central cylinder** (or **stele**), the inter-vascular ground-tissue itself being the **conjunctive tissue** of the stele. The outermost layer or layers of this stelar parenchyma, not separately distinguishable in the present case, will form the **pericycle,** which in other instances we shall become more clearly acquainted with.

Under the microscope with a low power we now look for a part of the section suitable for further study, choosing a vascular bundle which does not lie too near the periphery, because in this neighbourhood the structure of the bundle is simplified. In all cases it is necessary to note accurately in which direction the periphery of the stem lies, in order that we may know which is the inner and which the outer side of the bundle.

The vascular bundle which we select may appear somewhat as in the adjoining *Fig. 40.* We first notice the **Sheath** (*vg*), which surrounds the vascular bundle, and has become coloured reddish-brown in the chlorzinc iodine. It consists of strongly thickened and lignified **sclerenchyma fibres,** and has for that reason stained as noted above. It is more strongly developed on the inner and the outer edge of the vascular bundle, weaker upon its flanks. Working from the inner side of the bundle towards the outer, we next see at the inner edge an **intercellular passage** (*l*), surrounded by narrow cells, only slightly thickened, which are nevertheless coloured yellow by the chlorzinc iodine. Into this intercellular space projects a ring (*a*), belonging to an **annular vessel,** which has been torn by stretching. The intercellular passage, also, has arisen from the breaking down of cells. Such a method of development is expressed by the term *lysigenous*, whereas when the intercellular space arises only by the separation of the elements of a tissue, as in stomata, its origin is *schizogenous*. This torn vessel, together with some others, the thickening layers of which may perhaps also be seen projecting into the intercellular passage, represents the elements first formed in this part of the vascular bundle, elements which were developed at a time when the parts of the plant with which we are

now concerned were still in process of rapid elongation. Impinging on the intercellular passage on its outer side are one or more other vessels. They are recognisable by their cavity, which is larger than that of the neighbouring cells. In the bundle represented in Fig. 40, only one such vessel (*sp*), and that a rather narrow one, is present. These vessels, present to the number of one or more, are, as can be demonstrated only in longitudinal

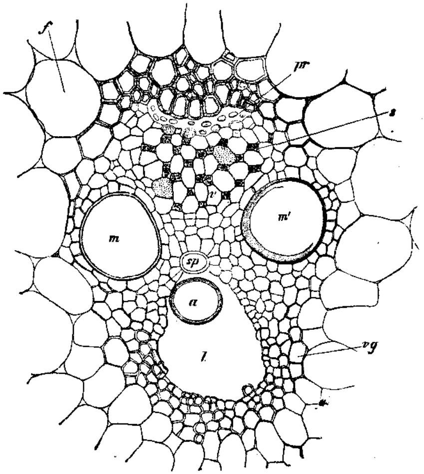


FIG. 40.—Cross-section through a vascular bundle from the inner part of the internode of the stem of *Zea Mais*. *a*, Segment of an annular vessel; *sp*, spiral vessel; *m* and *m'*, pitted ducts with unbordered pits; *v*, sieve-tubes; *s*, companion-cells; *pr*, crushed elements of the protophloëm; *l*, intercellular passage; *vg*, sheath (× 180).

section, thickened in a spiral manner. They are **spiral vessels.** Next, in each half of the bundle, right and left, is a wide cavity (*m*, *m'*). These are two vessels with pitted, rarely spirally reticulate, thickened walls. They are the so-called **pitted ducts.** Often in the cavity of these great vessels a ring, or part of one (*m'*), can be seen projecting as a thickening of the wall. This is the remains of a cross partition-wall which, diaphragm-like, is broken

through. It is this rupture of the partition-wall which enables us to distinguish such elements as vessels or tracheæ, in contrast to the pseudo-vascular or tracheïdal elements, the partition-walls of which are not broken through.[1] Where tracheïdes simulate tracheæ in appearance they can be distinguished as **vascular tracheïdes**, in contra-distinction to the fibre-like tracheïdes or **fibrous tracheïdes**.[2] Tracheæ and tracheïdes are only dead elements, without living contents, which serve for the conduction of water. The two large vessels in the vascular bundle of *Zea Mais* are either completely surrounded by flat parenchyma cells, or on one side they impinge directly on the elements of the sheath. Towards the sheath elements the vessels bear either no, or small, scattered, pits; while large pits connect them with the parenchyma cells. Between the two vessels the tissue consists of parenchyma, in which are scattered a smaller or larger number of tracheïdes. All these elements are coloured yellow-brown with chlorzinc iodine.

The part of the vascular bundle which we have thus far considered we distinguish as the vasal or vascular portion, also as the **wood** or **xylem**, also **Hadrome**. The parenchyma of this vascular part we can distinguish from other parenchyma by naming it the vasal parenchyma, or wood parenchyma. The wood parenchyma cells which immediately surround the vessels, which in their form and the structure of their walls betray a relation to the vessels, we can in particular regard as vasal covering-cells. The more or less destroyed vascular elements, which we met with in the inner edge of the vascular bundle, indicate the first elements which were developed in the vasal portion of the vascular bundle and served for the conduction of water during the elongation of this portion while still in the developmental stage. These can be distinguished as the primary wood or **protoxylem** elements.

On the outer side of the xylem strand the vasal parenchyma cells are followed by wide cavitied **sieve-tubes** (*v*) in regular alternation with their narrow cavitied **companion-cells** (*s*).

[1] To distinguish microscopically between a true trachea and a vascular tracheïde of the spiral or annular type is a matter of extreme difficulty; hence whether a particular vascular element is or is not a trachea is often in dispute, and for these elements of the spiral and annular type the terms trachea, and vessel (with a reservation as to their exact character) may be in general conveniently used.—[ED.]

[2] They might be known as "*fibroids*".—[ED.]

They serve for the conduction of proteids. This string of tissue is accompanied on the flanks by a layer of thin-walled cells; still farther outwards it is succeeded by a narrow layer of strongly swollen functionless sieve-tubes and companion-cells (*pr*). These last have taken a brownish colour in the chlorzinc iodine, while the other elements are violet. The companion-cells in the active portion are distinguished also by rich protoplasmic contents, stained yellow-brown. Here and there the cross-section may have skirted the end wall of a sieve-tube, which will then show the finely punctate **sieve-plate.**

To distinguish it from the vasal part, this portion of the vascular hundle, composed of sieve-tubes and companion-cells, is called the sieve portion; also by the names **Bast** or **Phloëm,** and likewise **Leptome.** For the entire vascular bundle, composed of xylem and phloëm portions, the term vascular bundle may be used, as also in some cases fibro-vasal or fibro-vascular hundle. In contrast to Hadrome and Leptome the collective term Mestome may also be used.[1] The swollen elements situated in the periphery of the sieve portion belong to the time of the first development of the bundle, when they were themselves newly developed, and active, and (just as with the protoxylem) they constitute the **protophloëm**, the first formed elements of the bast.

Such vascular bundles as this, in which the sieve portion impinges unilaterally upon the vasal portion, are known as **collateral.** As in the development of the hundle no tissue capable of division persists between the vasal portion and the sieve portion, such bundles are spoken of as **closed.**

The layer of thin-walled unlignified cells along the flanks of the bast appertains to the vascular bundle sheath; and in the same way the protopbloëm is bounded by sheath elements, but in this case thickened and lignified. These last are sclerenchyma fibres, but are distinguished by their exceptional width. The sclerenchymatous elements of which the sheath is composed pass over by a few intermediate elements (belonging, doubtless, to the ground-tissue, but the position of which has prevented expansion) into the large-celled parenchymatous ground-tissue (the **conjunctive-tissue**) of the central cylinder (or stele). The

[1] The terms "Vascular portion" and "Sieve portion" were given by De Bary; Xylem and Phloëm by Nägeli; Hadrome, Leptome and Mestome by Haberlandt and Schwendener. These last-named terms have a wholly physiological significance.—[ED.]

walls of these ground-tissue cells in the fully developed stem are also coloured yellow by the chlorzinc iodine, and only here and there show a shade of violet.

Approaching now the periphery of the stem we note that the vascular bundles become much more closely crowded together, their cross-section is smaller, the intercellular passage disappears, and a median row of vascular tracheïdes takes its place. In the outermost smallest vascular bundles both protoxylem and protophloëm are wanting, which is connected with the fact that these vascular hundles are first formed after the elongation of the internode has ceased. The two lateral vessels diminish in size; they are connected by others placed between them. The bast undergoes a specially marked reduction. On the other hand, the sclerenchyma sheath of the bundle increases in thickness, but only around the inner portion of the vascular bundle. In proportion as the sclerenchyma sheath increases in strength, the so-called "transfusion" places at the limits of the wood and bast show more and more clearly. At these places the vascular bundle sheath remains feebly developed, its elements unthickened. The transfusion places permit the exchange of nutrient materials between the interior of the vascular bundle and the surrounding ground-tissue. In the outermost vascular bundles, which have undergone the greatest reduction, the bast is seen to be sunk in the wood; the two transfusion places have joined on the outer side of the hundle, and form one common transfusion place; while the sclerenchymatous elements of the sheath are altogether wanting there.

Outside the central cylinder lies the **primary cortex.** Next to the epidermis of the stem comes a more or less strongly-developed ring of tissue, the elements of which have the same appearance as those of the vascular-bundle sheath, and show the same reaction with chlorzinc iodine. Such a distinct sheath of tissue bounding the epidermis is known as **Hypoderma.** This hypoderma is interrupted only under the spots where lie the stomata. The hypoderma and the sheath of the vascular bundle have alike to provide for the protection of the thin-walled tissue and for the general stability of this part of the plant, and are included amongst the elements of the mechanical system, as **Stereïdes,** while the tissue which they constitute forms the mechanical tissue system, the **Stereome.** In proportion as the stem needs to be secure against flexion, so must the mechanical appliance, the stereome,

be removed as far as possible towards the periphery. The crowded peripheral vascular bundles, provided alike on the side of the bast and of the wood with a strong cover of sclerenchyma, represent here a system of combined upright girders. The sheaths of sclerenchyma are the ties; the vascular bundles themselves are the "filling". The hypodermal cylinder of sclerenchyma strengthens this action, even when, as in this case, not very strongly developed. This hollow cylinder is mechanically to be considered as a combination of numerous "ties," arranged in a circle.[1]

It is desirable now to prepare *radial longitudinal sections* through the stem. To obtain these take a piece of the stem about $\frac{1}{4}$ or $\frac{1}{3}$ inch long, cut it in two longitudinally through the middle, and take the sections from the cut surface of either half.[2]

[1] I have felt unable to give a quite satisfactory translation of the above passage in the text, and will therefore endeavour further to illustrate it. Two sets of phenomena have to be mechanically provided for, the one affecting the stem as a whole, the other its separate vascular bundles. First, as to the stem as a whole. It has considerable weight to bear, its own and that of its leaves. It must therefore be mechanically constructed to resist crushing. It has to bear often very great lateral strains, from winds. It must therefore also be constructed to resist flexion. In both these respects it can be compared with a pier of a bridge, especially a cylindrical iron pier of a lofty railway bridge. To resist flexion this is made hollow, so as to throw all the strength to the outside; and, to aid in resisting crushing, it may be filled with concrete. Secondly, as to the individual vascular bundles. The sclerenchyma layers will help in the above purpose; but the bundles, being on the one hand water, and on the other hand food conduits, must be protected mainly from the lateral strains which would tend to crush their elements, make them "collapse," and so cease to function. This protection is the main duty of the sheath of sclerenchyma. Its being most thickened on the inner and outer side of the bundles, and taking thus the form largely of two arches concave to each other, makes its structure the most advantageous for its purpose, since all the lateral strains in such a cylindrical stem may be considered to be radial. To this we may add one more factor: the course of each of the vascular bundles from its lower to its upper extremity is usually not straight, but in the form of an elongated arch, the concavity outwards, they thus become akin to "struts". If they anastomose, or join together, as they do most beautifully in many cases, the mechanical analogy is still more complete, since they then resemble the network of connecting girders or tie rods with which all observers of iron bridges are familiar. I select iron bridges for this illustration, for in them, as in nature, the smallest amount of material is made to go the greatest possible way.—[ED.]

[2] An excellent method of preparing longitudinal sections of a stem, if moderately thin, is to take a piece about $\frac{3}{4}$ inch long, cut it nearly through

Do not be satisfied with a single section, as otherwise the chance of obtaining in the preparation a vascular bundle cut, as it must be, actually median is small. Such a median-cut vascular bundle can be recognised on examination of the section, in that it will show at the same time the intercellular passage, the spiral or annular vessels, and the bast. If the section is laid in chlorzinc iodine we shall note the violet coloration of the bast, and the

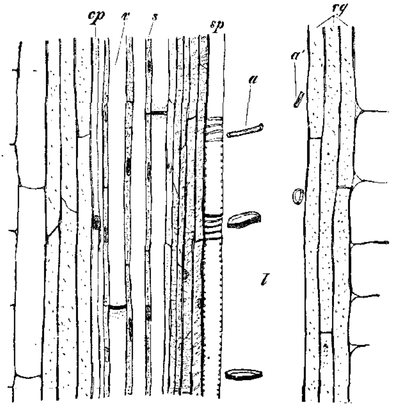


FIG. 41.—Longitudinal radial section through a vascular bundle in the stem of *Zea Mais.* *a* and *a'*, segments of an annular vessel; *sp*, spiral vessel; *v*, sievetube; *s*, companion-cells; *cp*, protophloëm; *l*, air passage (intercellular passage); *vg*, sheath (× 180).

thin-walled cells surrounding the intercellular passage have also a violet tone. The other elements, in accord with what we have seen in the cross-section, are coloured yellow to yellowish-brown. For further study we preferably select a section which we have stained with soda-corallin (Fig. 41). It is desirable first of all to determine in which direction in the section the surface of the stem lies. As in the cross-section, our examination will proceed

at a couple of points near the middle, and about ¼ inch apart, bend the ends down, using them as a handle to hold the material by, and take the sections then from the short median piece.—[ED.]

from the inner towards the outer edge of the bundle. We then see that to the broad cells of the ground-tissue, in outline well-nigh square, succeed narrower ground-tissue cells, and after these *follow the narrow elements of the vascular bundle-sheath* (*vg*). These last elements, deeply stained with corallin, show marked elongation, join one another with more or less inclined end-walls, and are provided with small, cleft-like, obliquely ascending pits. In the interior of each is to be found a lining layer of protoplasm of very reduced dimensions, and a small nucleus. From the nature of their pits we shall recognise these as sclerenchyma fibres, although they do not all possess the typical form of fibres, to wit, sharply tapering ends. To the cells of the sheath succeeds the intercellular passage, and we can determine that, unless the section is cut obliquely, it follows without interruption the whole length of the bundle. It is surrounded by thin-walled wood parenchyma, the cells of which are far shorter than the elements of the sheath, have more cell-contents, and have horizontal walls. Into the intercellular passage project usually isolated rings; they are attached to the outer side of the intercellular passage, *i.e.*, to the side nearest the periphery of the stem. *They arise from an annular vessel (vascular tracheïde)* torn during the elongation of the internode. Other smaller isolated rings may also often be seen clinging to this or the other side of the intercellular passage (*a*). Collectively they represent the remnants of the protoxylem. Impinging outwardly on the larger rings are one or several broader or narrower spirally or reticulately thickened vessels (vascular tracheïdes). In the case represented in Fig. 41, only one such was cut through, and that a somewhat narrow one (*sp*). Next follow comparatively short wood cells, with pitted or reticulately thickened walls, and between them also thickened tracheïdal elements. Thus we arrive at the bast or phloëm portion, recognisable in the corallin preparation by some thick rose-red coloured cross-walls, the **sieve-plates** of the sieve-tubes (*v*). These sieve-plates are highly refractive; and stronger magnification shows that they are pierced by fine pores, after the fashion of a sieve, and that on one side, seldom on both, is collected a highly refractive plug of "slime," due, however, to the method adopted in preparing the section. In the periphery of the bast (at *cp*), where in the cross-section were visible the swollen cell-walls of the protophloëm elements, a specially beautiful rose-coloured cross-wall appears.

This is a sieve-plate covered with Callus, the structure of which we shall study hereafter on other and more favourable objects. The callus absorbs corallin with special avidity, and hence the callus-plates are sharply stained. By the side of the sieve-tubes can be distinguished the companion-cells (*s*). They are narrower and shorter than the sieve-tubes, and have besides richly protoplasmic contents, and a readily visible nucleus, for which we look in vain in the sieve-tubes. Sclerenchyma fibres of the vascular bundle sheath, and with very oblique end-walls, bound the bundle towards the exterior. The innermost cells of the sheath have, as the cross-section has already shown, a comparatively broad cavity. Starch grains are not found in the cells of the vascular bundle; in this case, however, they are wanting in the cells of the ground-tissue also. All the cells of the vascular bundle and of the ground-tissue, with the exception of the tracheæ, tracheïdes and sieve-tubes, contain nuclei.

It is clear that such a median longitudinal section of the bundle as is described above, can show neither of the two great vessels—the pitted ducts. If the section be not very thin, one may show by deeper focussing, but it is indistinct. In order to study the longitudinal section of one of the great vessels, we must look for *a section which cuts the vascular bundle out of the middle line*. We shall then see that the great vessel is obliquely pitted, less often spirally or reticulately thickened. In these pitted ducts the thickened parts form a network. The pits broaden out at their bottom, but are, however, only unilaterally "bordered," in that the corresponding pit of the adjoining cell of the wood parenchyma is wanting in a "border". These cells, too, are less thickened than the vessels. The diaphragms, or cross-walls, of the great ducts at once attract attention in the longitudinal section. They show a double ring, which only projects a slight distance into the cavity of the duct. These rings originated in a thickening of the outer edges of the cross-walls, the inner unthickened part of which was afterwards dissolved (resorbed). From the number of the diaphragms we can therefore draw a conclusion as to the number and size of the cells of which the duct is composed. At the place of insertion of the diaphragm, the vessel shows a slight constriction.

Double Staining.[1]—It is very instructive to attempt double

[1] Stains used for cell-wall staining can be classified as those which stain (1) lignified membranes only, (2) unlignified membranes only, and (3) mem-

staining of cross-sections of *Zea Mais* with the object of bringing out its structure, each stain picking out some special structural feature. According to the stains used, the process can be by successive treatment with different stains, or simultaneous staining by means of a suitable mixture. We can obtain a very beautiful double staining if we allow the sections to lie for a little while in watery iodine-green solution and then for a somewhat longer time in Paul Mayer's alum-carmine. Instantaneous double staining can be obtained by means of picro-nigrosine or picro-aniline blue. In the carmine iodine-green preparation the unlignified membranes are stained with the carmine, the lignified walls by the iodine-green. In picric double staining the picrin dyes the lignified, the nigrosine and aniline blue the unlignified membranes. The fixed cell-contents have taken colour from the carmine, nigrosine or aniline blue respectively.

In some cases stained preparations which are to be preserved in glycerine-jelly should have the stain fixed, so that the glycerine may not dissolve it out. For amydo colours, such as methyl-blue or fuchsin, a mordant of tannin and afterwards tartar emetic should be used; for oxyazo colours, such as ponceau, a mordant of zinc chloride. In many cases a 10 per cent. solution of alum does well as a mordant. Many amydo-azo colours, such as benzo-purpurin, fix in an alkaline bath without needing any other mordant. What mordant to use in any special case, producing no "muddiness," but clear differentiation, should always be first determined.

Permanent double staining can be obtained by the use of methyl-violet and carmine. The sections are first treated for a few minutes with a fully concentrated alcoholic solution of

branes both lignified and unlignified, but with different degrees of intensity or in different tones or shades of colour. If permanent preparations are desired, important limitations arise from the relative durability of colours in the various mounting media. The list of permanent stains is small. Sometimes a single stain will do all that is necessary, in which case the student may restrict himself to the use of safranin, which differentiates tissues very well, the lignified membranes more cherry-red, the unlignified membranes more brown-red; and the staining is permanent. Fuchsin (magenta) also is very valuable as a fairly permanent stain for lignified membranes. If two stains are used consecutively, it is generally desirable to use first that which stains the lignified membranes; wash the preparation then in water (or in some cases alcohol) as long as is necessary, and then treat with the second stain.—[ED.]

methyl-violet, then so far decolorised in alcohol that the unlignified membranes appear only feebly stained, then transferred to water for a few minutes, and from this into one of the carmine solutions already mentioned, which will stain the unlignified membranes and protein cell-contents. Some control is needed in order to see when the staining has been rightly carried out, and the decolorising is best effected on an object-slide under the low power of the microscope. If the tiny white earthenware saucers sold for paint boxes are used for staining and decolorising, the white background much facilitates the process to the naked eye; or it is performed even more readily in a watch-glass supported upon a wire ring stand over a piece of mirror which will reflect the light through the staining or decolorising fluid. Very effective double staining can likewise be obtained by first staining with magenta dissolved in 50 per cent. alcohol, and then carefully removing the colour by means of alcohol until it is left only in the lignified and cuticularised membranes; and afterwards staining with logwood. For a beautiful double stain "Solid green" and Delta purpurin may be recommended; the vessels are stained deep green, the unlignified tissue red. An instructive double staining is also given by chrysoidin with azurin or purpurin.

Making Permanent Preparations.—It may be of interest to put up selected cross and longitudinal sections of the stem of *Zea Mais* as **permanent preparations.**[1] Neither the colouring due to chlorzinc iodine nor to soda corallin is permanent, and the double stains which we have tried also fade more or less in time, carmine and logwood alone being fairly permanent. Staining with aqueous solution of safranin can, however, be highly recommended, as the colour is quite permanent. As safranin gives beautifully differentiated specimens, the different cell-membranes taking on varied tones of colour according to the degree of their lignification, and the other differences in their constitution (*e.g.*, lignified walls cherry-red, unlignified walls brown-red), the preparations treated in this way are very instructive. A beginner might even with advantage restrict himself to this one method of staining permanent preparations.

Magenta (fuchsin) stains are also fairly permanent. The sections are laid for a time in a watery solution of magenta, and then washed with a solution of picric acid (1 part saturated alcoholic solution of picric acid to 2 parts water), and afterwards well washed with alcohol. The lignified cell-walls are stained an intense red.

[1] See note on page 120.

The sections stained with safranin, methyl-green carmine, or fuchsin-logwood, can be enclosed in glycerine-jelly. *Chemically pure* glycerine is also an excellent medium in which to enclose these preparations, but needs the edges of the cover-glass properly sealed. This can be done with a solution as thick as treacle of Canada balsam in chloroform, benzol, xylol or turpentine,[1] or gold-size may be used if the cover-glass edge is quite clean, for gold-size does not cling to a surface damped with glycerine. After laying on the cover-glass, any glycerine which flows out should be carefully removed with blotting-paper, and the closing medium laid in a streak round and over the edge of the cover-glass by means of a thin glass rod which has been dipped into it. This should be laid on in only a thin layer, and should not much overlap the cover-glass. To enable a preparation mounted in or closed with Canada balsam to be subsequently examined with a homogeneous immersion lens it is desirable to cover the edge, after it is quite dry, with a thin layer of gold-size, for which purpose a fine camel-hair brush is most suitable. The gold-size should be laid on very thin, and a second layer if necessary. Gold-size is not affected by the immersion oil, while Canada balsam is. Finally it is well to exercise judgment in the use of all mounting fluids, carefully proportioning the size of the quantity taken to the size of the cover-glass used and the apparent thickness of the section, *i.e.*, to the space which the mounting fluid has to fill. With practice this is by no means difficult, and it is possible to put up section after section without any overflow of the mounting fluid at all. If the amount necessary be *slightly* under-estimated, a little pressure on the cover-glass may effectively compensate.

For the removal from one fluid to another of large sections, the edges of which readily fold over, we can use, though with care, small section lifters of platinum, or of beaten copper wire, such as are recommended in the Introduction.

The stem of the Oat (*Avena sativa*), or of some other grass, can be taken instead of *Zea Mais*, should this latter not be available. In all these cases the vascular bundle will be found

[1] Of these the solution in turpentine is best, as it does not become brittle when dry; otherwise a jerk may make the cover-glass spring. The solution can be kept in a bottle with a bell-shaped external ground cap, to keep out the air.—[Ed.]

to have approximately the same structure. Either of these plants can, of course, be readily grown for use.

Vascular Bundle in Iris.—Now take *cross* and *longitudinal sections* of a fully-developed leaf of *Iris florentina*, or other Flag Iris, preference being again given to alcohol material, because it is more easy to obtain good sections, it contains no air, and besides this, the cell-contents are fixed, so that we can more readily study them. Section-cutting will be facilitated if the material is previously laid in a mixture of alcohol and glycerine. Lay the sections for a few hours in horax-carmine; then treat them for a short time with methyl-green. The cell-contents have taken up carmine, which in the form of borax-carmine does not stain the cell-walls, while the lignified walls are stained green with methyl-green. The vessels appear stained green, and usually also the outer elements of the sheath, *i.e.*, those impinging on the hast of the bundle. Besides this, a group of elements with swollen walls, the protophloëm, are noticeable in the outer region of the bast through their blue colour. We will therefore commence with the study of such a preparation, from which Fig. 42 is drawn. In this figure all the cells which are especially rich in protein contents, and therefore are conspicuous from their carmine colour, are shaded; the green stained walls of the vessels are, on the other hand, represented darker in the figure; while the protophloëm elements coloured blue are left clear. The thickened elements of the ground-tissue bounding the bast, when the section is taken through the base of the leaf, are still unlignified, and therefore remain unstained.—To rapidly stain a section, it can be treated with methyl-green alone; the colour of the cell-contents as here described is then absent. If the methyl-green is to stain only the lignified cell-walls, the exact time for staining must be carefully watched, or, if over-stained, the cellulose walls of the ground-tissue can be decolorised by rapidly washing in alcohol, best under the low power of the microscope, stopping the process at the right moment by transferring the section to water.—We proceed to examine the bundle from the wood towards the bast, and therefore from the upper surface of the equitant leaf, turned inwards, towards the lower surface, turned outwards. We first note that the number of vessels, or vascular tracheïdes, in the wood is pretty considerable, and that their width diminishes towards the bast. The vessels are in direct contact with one another, or else are separated by the slightly thickened compara-

tively narrow cells, with abundant cell-contents, of the primary wood parenchyma. Similar cells also surround the vessels on the flanks of the bundle, and separate them from the ground-tissue (shaded cells in Fig. 42). At the inner margin of the wood are always to be seen some crushed elements, the protoxylem (*ss*), whose walls are stained like those of the vessels. The bast again shows an alternation of larger and smaller cells; the contrast is here, however, not so striking, nor is the regularity of arrangement so great, as in *Zea*. The cells with broader cavities are the sieve-tubes, the smaller ones, marked by their abundant cell-contents, the companion-cells. In the outer region of the bast lie the crushed proto-phloëm elements (*pr*), to which we have already referred, which have ceased to function, and which have swollen walls more or less deeply stained blue. This outer bast portion is enclosed by the strongly thickened sclerenchyma of the sheath, which supports the vascular bundle with a more or less strongly developed strand. Around the remainder of the vascular bundle

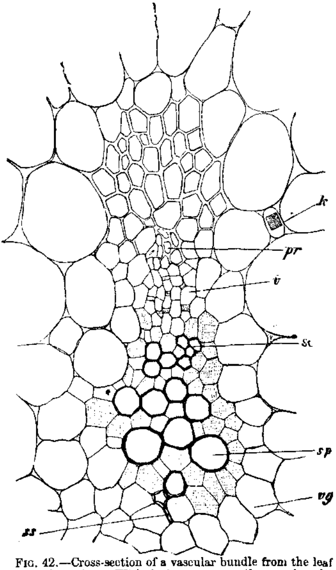


FIG. 42.—Cross-section of a vascular bundle from the leaf of *Iris florentina*. With dark contour are the vessels; the cells of the bundle which are rich in contents are shaded. *ss*, crushed protoxylem; *sp*, broader spiral vessels or tracheïdes; *sc*, scalariform vessels or tracheïdes; *v*, sieve-tubes, between which are the narrow companion-cells; *pr*, crushed proto phloëm elements; *vg*, sheath with wavy radial walls; *k*, section through a crystal (× 240).

a sheath is not clearly defined; yet it can be seen that the cells of the ground-tissue nearest to the vascular bundle are smaller, and that they join together without a break.

By a scrutiny of the tissue in the neighbourhood of the vascular bundle it will be seen that single small cells, between the large cells of the ground-tissue, contain a highly refractive crystal (Fig. 42, *k*). It offers itself to us here in cross-section, or end view; as to its longitudinal form we can readily inform ourselves by suitable sections. Similar crystals, contained in narrow cells, can likewise be seen here and there interposed amongst the larger cells of the general tissue of the leaf.

A *longitudinal section* through the leaf, cutting through the middle of a bundle, shows us at the inner limits of this bundle the greatly stretched, partly crushed, spiral vessels (or vascular tracheïdes) which we saw in cross-section at *ss*, and distinguished as protoxylem elements, *i.e.*, as the first developed elements of the wood portion of the bundle. Following are broader more closely wound spiral vessels (or tracheïdes), and then narrow elements with scalariform thickening. In the bast the sieve-tubes show clearly only when the sieve-plates have been stained with corallin. Farther outwards the sclerenchyma fibres are recognised by their strong thickening, their notable length, and pointed ends.

In order to control the results hitherto obtained, take some cross-sections from a living leaf. We then note that the large cells of the ground-tissue in the outer parts of the leaf contain chlorophyll grains, while the cells sheathing the vascular bundle are devoid of chlorophyll. In fresh preparations the vessels are filled with air, whence the structures are less clear than in alcohol material.

Crystals in Iris.—As the crystals are directed parallel to the long axis of the leaf, they show in profile in *longitudinal sections* (Fig. 43, *A*, *D*). They lie in elongated cells of the ground-tissue, which are only a little larger than the crystals themselves. These cells contain no chlorophyll, while the neighbouring cells usually contain chlorophyll. The crystals in question dissolve readily if treated with hydrochloric acid, without evolution of gas; whence we readily conclude that they are composed of oxalate of lime. All the crystals occurring here have an elongated prismatic form, and belong to the monoclinic system; most of them appear n-·minate (twin crystals), (*D*).

The vascular bundles of the Monocotyledons, if we exclude immaterial modifications, reductions, and amalgamations, are constructed upon the type of the two cases we have here studied, and we need not therefore further study them.

Thickening Stem of Dracæneæ.—Closed vascular bundles are not capable of increase in thickness, and therefore in the comparatively few cases where such occurs in the Monocotyledons, it cannot be brought about through the medium of the vascular bundles themselves. This increase of thickness results from the action of a **Cambium-ring** which is found external to the vascular bundles; and is almost wholly confined to the families of Dracæneæ and Aloïneæ, *i.e.*, the so-called "Arborescent Liliaceæ," tbe Dioscoreæ, and some of the Palms.

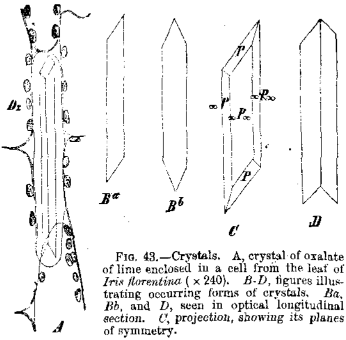


FIG. 43.—Crystals. A, crystal of oxalate of lime enclosed in a cell from the leaf of *Iris florentina* (× 240). *B-D*, figures illustrating occurring forms of crystals. *Ba*, *Bb*, and *D*, seen in optical longitudinal section. *C*, projection, showing its planes of symmetry.

For their study we select as a favourable object the plant so commonly cultivated in gardens and nurseries as *Dracæna* (more properly, *Cordyline*) *rubra*, but any available species will equally serve. The plant must be sacrificed for the purpose of the investigation. Examine the cut stem first with the naked eye; we shall notice externally a brown cork-layer and inside it the green soft cortex, somewhere about $\frac{1}{25}$ inch thick, and inside this the yellowish hard tissue of the central part of the stem. Between the two latter tissues lies the cambium-ring, which can be distinguished from the general yellowish tissue of the central cylinder as a lighter coloured ring.

We now submit a *cross-section* to microscopical examination, and first with a low power (Fig. 44). We then see, first, in the central (stelar) portion of the stem, a ground-tissue composed of

rounded cells (*m*), the conjunctive parenchyma of the stele, in which are irregularly scattered isolated circular or elliptic vascular bundles (*f'*). Outside a definite position (the inner *f''*) the bundles are more numerous, elongated in radial direction, and crowded so closely together that they appear separated only by comparatively thin streaks of ground-tissue. In these latter the cells are more strongly thickened, coarsely pitted, more or less elongated in the direction of the radius, and clearly arranged in radial, often wavy, rows. At the outer *f''* this ceases, and then we come to the boundary (*c*) between the yellowish central cylinder and the green cortex. We find here a zone composed of flattened thin-walled cells, strictly arranged in radial rows. It is the **Cambium-ring,** which provides for the increase in thickness of the stem. It belongs in origin to the peripheral ground-tissue of the central cylinder, or stele; to the region which we know as the **pericycle.** Its cells are in active division, and produce on their inner, and to a much smaller extent their outer, side new elements. These divisions result from the formation of tangential walls, and produce therefore radially arranged cell-rows, which from time to time are made double by the formation of radially-directed walls. Embedded in the young

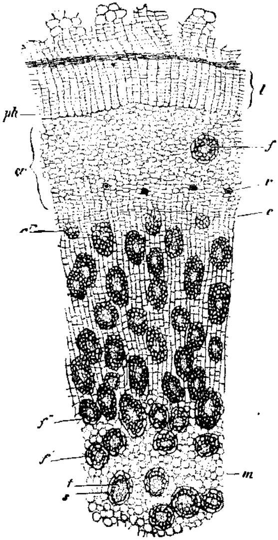


FIG. 44.—*Dracæna* (*Cordyline*) *rubra*. Cross-section through the stem. *f*, vascular bundles, *f'* being primary, *f''* secondary, and *f'''* leaf-bundles; *m*, unlignified elements of the ground-tissue; *s*, lignified elements of the ground-tissue, sheathing the vascular bundles; *t*, tracheïdes; *c*, cambium-ring; *cr*, cortex; *l*, cork; *ph*, cork-cambium; *r*, bundles of raphides (× 30).

growing tissue produced on the inner side of this cambium-ring are numerous vascular bundles in all stages of development. The youngest consist of a group of thin-walled cells, the oldest are already perfect at their inner edge, while the thin-walled outer edge, *i.e.*, the portion towards the periphery, is still immersed in the cambium-ring and in course of development (the outer f''). From the position where the vascular bundles appear crowded together, and the cells lying between them have acquired a radial arrangement, the tissues are **Secondary tissues** produced by the activity of the cambium-ring. External to the cambium-ring, and also resulting from its activity, is the **Secondary cortex** (*cr*), consisting of rounded cells. Between these, especially in the inner part of this cortex, occur single cells, in which lie fine needle-like crystals, closely packed together into a bundle (*r*). These are the bundles of so-called **Raphides**, composed of oxalate of lime. They are here seen in end view. Individual raphides-containing cells are sure to be opened by the razor in cutting, and the fine needles will therefore be found lying scattered over the section. The rest of the cortical cells contain chlorophyll grains. In the cortex are also visible occasional circular cross-sections of bundles (f''') which are passing outwards into the leaves. They are the so-called **leaf-traces.** Next to the secondary cortex comes a thick layer of thin-walled, colourless cells, arranged radially (*l*), which on its outer side passes over into a brown, less regular tissue. This is the **Cork-layer**, consisting of colourless developing cork-tissue in its more internal, but of old irregularly elongated and discoloured cork-tissue in its outer parts. The nature and mode of development of cork we will study hereafter.

If a thin section is treated for some time with chlorzinc iodine very beautifully differentiated figures are obtained. Examined with a high power the rounded ground-tissue elements have violet walls, on which the numerous pits show as colourless spots. In the **vascular bundles** of the central part of the stem the centrally placed bast is stained violet, the surrounding xylem is yellow-brown to a muddy-brown. The bundles are concentric, with external wood; a condition much more common in rhizomes than in aerial stems. The bundle is surrounded by a generally double layer of lignified elements stained brown, and with simple rounded pits; these form the sheath, and make the bundle air-tight to the ground-tissue. In the vascular bundles of cambial

origin the wood consists only of tracheïdes with bordered pits surrounding a very reduced bast; the radially-arranged ground-tissue cells between the bundles may be considered as medullary rays, *strictly comparable with those of Dicotyledons and Gymnosperms.* The whole of the secondary ground-tissue is stained brown. The bundles often anastomose tangentially. The cells of the cambium-ring are swollen by the reagent and stained violet. Clearer spots on the ring indicate more swollen but less stained rudiments of the vascular bundles. The rounded cells of the cortex are violet, the cork-cells yellow-brown, but the innermost layer, the cork-cambium, is also violet.

NOTE ON THE TEMPORARY CLOSING OF MICRO-PREPARATIONS (p. 112).

Preparations lying in a fluid capable of evaporation, which it is desired should be accessible for further manipulation, may be temporarily closed by means of wax. A wax vesta, or taper, is lighted and blown out, or merely warmed over a flame, and while still warm, four spots of wax are made on the four corners of the cover-glass in order to fix it in position, and the wax then drawn round the edge till it is completely closed. This form of closure can be very easily removed.

NOTE ON PERMANENT PREPARATIONS IN GLYCERINE-JELLY (p. 113).

Glycerine-jelly contracts considerably in drying, and the cover-glass is then liable to "spring". To obviate this, after eight months or so have elapsed, a narrow and thin layer of gold size can be laid, with a fine brush, over and round the edge of the cover-glass. This will also run in under the cover, fill up any air spaces, and make a close and air-tight adhesion between cover-glass and micro-slide. This also serves to exclude moulds, which otherwise may ruin a preparation, even after years have elapsed.

CHAPTER IX.

OPEN COLLATERAL VASCULAR BUNDLES — LATEX — BICOLLATERAL BUNDLES—STRUCTURE OF SIEVE-TUBES—THE THICKENING OF DICOTYLEDONOUS STEMS.

Principal Materials Used.

Runners of *Ranunculus repens;* fresh, or in alcohol.
Stems of *Chelidonium majus;* in alcohol.
Roots of *Scorzonera;* fresh, or in alcohol. Or, Dandelion.
Stems of *Euphorbia splendens*, or *E. jacquinæflora;* in alcohol.
Stems (not young) of *Bryonia dioica*, or other Cucurbit; in alcohol. Also fresh.
The same fixed with boiling water and preserved in alcohol.
Twigs of *Aristolochia Sipho*, ⅛ to ¼ thick; also thicker twigs; preferably in alcohol. Or, young plants of *Ricinus communis*, *Helianthus annuus*, or *H. tuberosus*.

Principal Reagents Used.

Chlorzinc iodine—Potash—Aniline blue—Soda corallin—Sulphuric acid—Phloroglucin and hydrochloric acid.

Stem of Ranunculus repens.—As a first example for the study of the **open collateral vascular bundles**, which are peculiar to Dicotyledons, we select the creeping stems (runners) of *Ranunculus repens*, the creeping Buttercup. The *cross-section* shows that the vascular bundles are separate from one another, but arranged in a simple circle, so that the **ground-tissue**, as a whole, shows a central portion, or **pith**, an external portion, or **cortex**, while between the bundles are broad strips of ground-tissue which we may call the **medullary rays**. This distinction we will discuss in greater detail hereafter. The ground-tissue, generally, consists of rounded cells, which become smaller towards the periphery of the stem, contain chlorophyll grains, and have between them large intercellular spaces. The epidermis forms

the surface of the stem ; in the interior, through the stretching apart and destruction of the cells of the central portion of the pith, the stem is hollow. The vascular bundle gives the same general impression as that of the Monocotyledons ; the same parts are recognisable in the same order. The xylem portion at

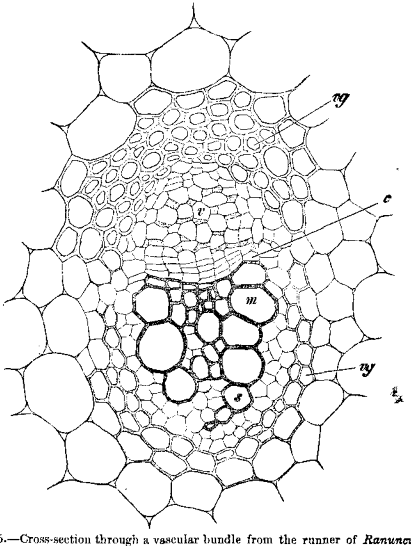


FIG. 45.—Cross-section through a vascular bundle from the runner of *Ranunculus repens*. *s*, spiral vessels ; *m*, vessel with bordered pits ; *c*, cambium ; *v*, sieve-tubes ; *vg*, sheath (× 180).

its inner edge consists of thin-walled wood parenchyma, in which are a few more or less disorganised protoxylem elements ; then fol-w gradually broadening spiral vessels, or vascular tracheïdes (Fig. *s*). Farther outwards succeed vessels with obliquely elongated ›nd then the same with typically constructed bordered pits at is, in which the pit broadens out to its base, so

that its diameter is greatest at the closing membrane, a kind of chamber being thus formed in the thickness of the wall, so that they might be called "chambered pits". The comparatively narrow vessels in the median portion of the bundle form a connected group in which wood parenchyma is completely wanting.

Outside the xylem is to be found a tissue composed of several layers of flat, unlignified, thin-walled cells, arranged in straight radial rows. This is a growing layer, or **cambium**, of, in this case however, strictly limited activity. This is our first acquaintance with a cambium layer as a constituent portion of a vascular bundle by means of which xylem and phloëm can increase in quantity; and from its presence these are called **open bundles**, *in contradistinction to the* **closed bundles** *we have studied in* Monocotyledons. Outside the cambium is the readily recognisable **phloëm** or bast, which closely resembles that of *Zea Mais*, and, as in it, is composed of sieve-tubes (*v*) and companion-cells (shaded in figure) in fairly regular alternation. Most externally a few protophloëm elements are found. A **sheath** (the upper *vg*) of sclerenchyma fibres surrounds the vascular bundle, and is specially strongly developed, in the form of an arc, outside the bast. *At the outer edge of the phloëm it is in direct contact with* the protophloëm, but, as in *Zea Mais*, a layer of unthickened cells, belonging, however, to the sheath, separates the bast laterally from these thickened elements. Similarly upon the inner or xylem edge of the bundle, the sheath forms a deeper but thinner arc (the lower *vg*) separated from the wood by unthickened parenchyma. Between the two arcs of the sheath, opposite, that is, to the ends of the cambium layer, the sheath is apparently wanting; these are places of passage or **transfusion**, by which communication for water or food stuffs is maintained between the vascular bundle and the surrounding conjunctive parenchyma. They are composed of cells which are more feebly thickened and only slightly or not at all lignified, and show up particularly clearly, therefore, in the preparation if it is examined in chlorzinc iodine. This vascular bundle, that of an undoubted Dicotyledon, is therefore comparable in a quite marked degree, with that of a typical Monocotyledon like *Zea Mais*.

Let us now take *radial longitudinal sections* through the runner, preparing them in the manner already recommended, *i.e.*, by nearly cutting a piece of the runner through at two points

about $\frac{1}{8}$ inch apart, bending down the ends as a handle, and cutting sections through the short middle portion; or, take a piece about $\frac{1}{5}$ inch long, stick a needle through it near one side, hold it thereby flat on the index-finger, and cut sections. If the section cuts radially through a vascular bundle, as it should do, we can see in it, passing from the inner edge outwards, the narrow annular and spiral vessels (vascular tracheïdes), then the pitted ducts, between them elongated wood parenchyma, then the thin-walled elongated cambium cells, the sieve-tubes and companion-cells, and at both edges the sclerenchymatous elements of the sheath. If our section is laid in chlorzinc iodine we shall notice reddish grains in the sieve-tubes. These are starch grains which contain amylo-dextrin, and which the sieve-tubes appear in this case to be richly provided with.

Latex-vessels of Chelidonium.—The vascular bundle of the Great Celandine, *Chelidonium majus*, is so similarly constructed to that of *Ranunculus repens*, that the *cross-section* will be at once understood. Assuming that our section is from alcohol material, we shall note in it, however, many cells with dark-brown contents in the bast of the bundle, on the inner limits of the xylem, and in special number on the flanks and at the outer edge of the arc of sclerenchyma which covers the bast portion of the vascular bundle, and singly also in the ground-tissue between the bundles. These contents consist of the orange-red **milk** or **latex** of *Chelidonium*, coagulated in alcohol, and the cells in question are so striking that it is impossible to overlook them. They are all thin-walled, even those which are inserted in the outer edge of the sclerenchyma string; they are not otherwise distinguished by any special form.—These **latex-vessels** or **laticiferous-vessels** can be found very easily also in *radial longitudinal sections*, being recognised at once by their yellowish-brown contents. They present the appearance of long tubes running approximately parallel to the long axis of the stem. Without difficulty the existence of cross-walls in these tubes can be determined. These cross-walls are more or less clearly pierced in their centres by one or several pores; they are wanting, however, here and there, where we should expect to find them, since they may have been completely dissolved. Lateral communications of the latex-vessels cannot be observed in *Chelidonium*.—If the stem used be at all thick, the latex-vessels can be even more readily found in *longitudinal tangential sections*, in which, by reason of their

distribution, they are likely to be much more numerous than in the radial section. The facts thus made out exactly tally with those just detailed, and serve to supplement them.

Laticiferous Network.—**Anastomosing latex-vessels**, in which the latex system is converted into a whole, by means either of the perforation of connecting cells, or the putting forth of lateral anastomosing protuberances, can, however, be found in the Poppies (*Papaveraceæ*), the Bellworts (*Campanulaceæ*), and in the liguli-floral section of the Composites (*Cichoriaceæ*), as, *e.g.*, in the Dandelion. Of these we can select as an example the garden *Scorzonera* (*S. hispanica*), not infrequently grown in kitchen gardens for its parsnip-like roots. *Tangential sections*, taken from the external part of the root, a short distance below the exterior, if treated as described above, will show in the bast portion of the vascular bundles an extensive network of latex-vessels, filled with their very granular contents, shown in Fig. 46. A somewhat similar network, though not so clearly displayed, is shown in the root of the Dandelion, by means of tangential sections, which are best cleared by treatment with potash.

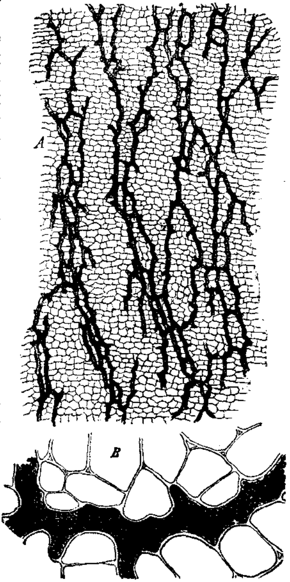


FIG. 46.—Latex vessels in the bast of *Scorzonera hispanica*, tangential section. *B*, a portion of *A*, more highly magnified. (After Sachs.)

Latex-cells of Euphorbia.—In the Euphorbiaceæ, and a few other plant groups, the latex is found in enormously elongated,

branched, but not anastomosing, cells of which we have already (p. 19) studied one of the contents in the form of peculiar starch grains. These **latex-cells** are best studied in the stems of one of the semi-succulent greenhouse Euphorbias, such as *E. splendens* or *E. jacquinæflora*. *Tangential sections* through the thick cortex, especially if treated with iodine, show fairly regular parenchymatous cells, and amongst them the latex-tubes, recognisable from their moderately thick walls and contents. The walls, as chlorzinc iodine will show, are of cellulose, smooth and elastic; they have a lining layer of cytoplasm, in which are numerous nuclei; otherwise are filled with a colourless, apparently granular latex enclosing the starch grains referred to. By careful maceration these branched unsegmented latex-tubes can be extracted free, and to a considerable length.

Bicollateral Bundles of Bryonia. Sieve-tubes.—In almost all the Cucurbitaceæ, and certain other Dicotyledonous groups, we find isolated vascular bundles, with two bast strings, one on the outer, the other on the inner, side of the wood. These vascular bundles are therefore constructed **bicollaterally.** The outer bast is separated from the wood by a cambium of limited activity, the inner bast impinges immediately upon the inner limits of the wood. If the white Bryony of our hedgerows (*Bryonia dioica*), the only British cucurbit, is available, it will serve admirably for the study of the stem; otherwise recourse must be had to one or other of the cultivated varieties of *Cucurbita Pepo,* such as the cucumber, or vegetable marrow. The stem-part used must not be from anywhere near the growing point, or the large and very striking ducts will not be fully formed, nor the sieve-tubes completely developed; material had best be taken, therefore, from the more mature, discoloured parts of the stem, and preserved in alcohol. The **vascular bundles,** as examination of the *cross-section* (which has been stained in aniline blue and mounted in glycerine) with a low power, or even with the naked eye, will show, are arranged in two concentric rings, usually five in each ring. Those in the outer ring are small, and placed opposite the angles of the stem; those of the inner ring are much larger, and alternate with the outer ones. The protection of the inner parts of the stem is subserved by a **pericyclic ring of sclerenchyma fibres,** the elements of which have stained far more deeply than the large-celled ground-tissue. Externally to this ring, and separated from the central cylinder, or stele, by a layer of cells,

rich in starch, the so-called **starch sheath**, is a cortical parenchyma, which in the fresh stem contains chlorophyll; and then an interrupted ring of quite typical shining colourless **collenchyma.** At the interruptions in the collenchyma ring the inner chlorophyll-containing cortex extends to the epidermis, which has stomata at these places, by means of which gaseous interchange with the inner cortex is maintained. In the interior the stem is hollow.—Treated with iodine the starch sheath shows up very clearly, and the protective ring of sclerenchyma is very deeply stained. The individual vascular bundles have no protective sheath, and are not sharply delimited from the surrounding tissue. The parts appertaining to the vascular bundles are, however, more deeply stained than the ground-tissue. If we imagine the inner bast removed, the figure approaches as nearly as possible to the structure of the dicotyledonous bundle of *Ranunculus* and *Chelidonium.*

Choose a vascular bundle in which are two exceptionally large ducts, as fully developed as possible. If we commence our examination at the outer limits of the inner bast, we shall see at the inner edge of the xylem the crushed **protoxylem,** scattered in the thin-walled, unlignified, sometimes chlorophyll-containing, xylem parenchyma. Then follow, outwards, annular and spirally thickened vessels (or vascular tracheïdes), successively increasing in diameter; then spiral vessels with close spirals, then, in the median portion of the bundle, pitted ducts, some very narrow. The two large ducts on the flanks of the vascular bundle have bordered pits, and are surrounded by flat wood parenchyma; similar parenchyma surrounds the median group of narrow pitted ducts. Upon this xylem parenchyma impinge externally the radially arranged cell-rows of the **cambium**; these pass over into the active elements of the **bast.** This latter consists of broad sieve-tubes, each accompanied by one (rarely two) narrow companion-cells, and of ordinary bast parenchyma, broader than the companion-cells. This bast parenchyma increases proportionately towards the exterior, where is found the highly refractive protophloëm. The inner bast string is constructed exactly as the outer, but in inverse order. It overlaps the inner edge of the xylem like the horns of a new moon. The two are separated by radially-arranged thin-walled parenchyma, which, however, must not be looked upon as a cambium, since it has early lost its capacity for growth and become permanent.—In the sieve-tubes of Cucur-

bitaceæ the end **sieve-plates** are placed horizontally; hence in both portions of the bast they will probably be exposed in full-face view. They are readily recognisable from their areolation (Fig. 47, *A*) and high refraction. The small companion-cells (*s*) will also be recognisable from the density of their plasmic contents. If a section is treated with soda corallin, the sieve-plates show up very clearly with a rosy colour; if treated with aniline blue they are deeply stained by it.

Radial longitudinal sections, which have cut through a vascular bundle, show the tracheïdes and vessels with their

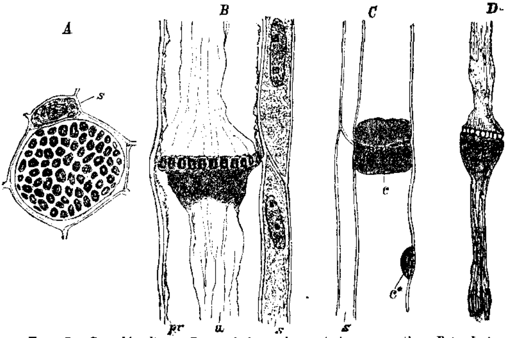


FIG. 47.—*Cucurbita Pepo.* Parts of sieve-tubes. *A*, in cross-section, *B* to *D*, in longitudinal section. *A*, a sieve-plate seen in full face; *B* and *C*, side-view of the adjoining parts of two sieve-tubes; *D*, the connected parts of the slime-strings of two sieve-tubes, after the action of sulphuric acid; *s*, companion-cells; *u*, string of slime; *pr*, protoplasmic lining layer; *c*, callus-plate; *c**, small unilateral callus-plate from a lateral sieve-plate (× 540).

characteristic thickening, and that the two big ducts are constructed of very short barrel-shaped segments. Their pits are bordered; those of the xylem parenchyma are unbordered (simple).

Structure of the Sieve-tubes.—Longitudinal sections of alcohol material will enable us to study the unusually large **sieve-tubes.** They are long continuous sacs, the successive segments of which are separated by horizontal **sieve-plates** (Fig. 47, *B*). Study will be facilitated if we lay the longitudinal section for a short time in aniline blue, and afterwards examine in glycerine. After

lying for a fairly long time in this latter, the cell-walls are more or less completely decolorised, while the contents of the sieve-tubes retain the stain. Almost all the sieve-plates are placed horizontally; only a few have an inclined position. Most of them appear covered with a highly refractive callose substance, and show, proportionally to this, a not inconsiderable thickness (Fig. 47, *C*), so that they are evident with a low power. In our aniline-blue preparations these callose sieve-plates are coloured clear blue. In the interior of the sieve-tubes is visible a contracted, sac-like, axial string (*u*). This is a string of protoplasmic slime or mucus, which broadens at its ends, and almost completely covers the sieve-plate. It is stained indigo blue. One of the ends adjoining the sieve-plates is usually more densely full of contents, which form a terminal "slime-plug". Besides the axial sac, the sieve-tube shows with very careful examination, a thin lining layer of protoplasm, extremely thin, and adhering in most places closely to the wall of the sieve-tube. A nucleus is not present. In somewhat younger sieve-tubes the slime-string can be often seen to protrude through the pores of the sieve-plate from one constituent cell of the sieve-tube towards the other in the form of bladder-like or worm-like prolongations. In older sieve-plates such prolongations can no longer be seen; the callose substance has augmented, and contracted the sieve-pores, but throngh these contracted pores the slimy contents of one constituent cell of a sieve-tube are continuous with those of another (as in *B*). At the outer and inner edges of the vascular bundle the sieve-plates, covered with a callus layer, are very noticeable (Fig. 47, *C*). The callus-plates are clearly distinguishable from their high refraction, and are stained sky-blue. In the mid-thickness of the callus-plate the sieve-plate is more or less clearly recognisable. The callus-plate consists, therefore, of two halves, belonging to adjoining cells of the sieve-tube; fine pores, which ultimately are only recognisable as delicate striæ, traverse the callus-plate and the pores of the sieve-tube. Where two sieve-tubes are in lateral contact, small sieve-plates are formed on the common side-walls. These also later on possess a unilateral (c^*) or bilateral callus-plate, and are thus very visible. By the side of the sieve-tubes are the **companion-cells** (*s*); they are much shorter, so that four or five correspond with the length of a segment of the sieve-tube. The study of their development shows that a companion-cell arises originally as sister-cell to a

member of the sieve-tube, by longitudinal division of a single cell, and that this original companion-cell divides by repeated cross-divisions into a row of superposed companion-cells. They have abundant protoplasm and a large nucleus, and are connected with their sieve-tubes by elongated pores. The companion-cells of the successive segments of a sieve-tube often do not join on to one another. Amongst the sieve-tubes and companion-cells are elongated thin-walled bast parenchyma.

It is very instructive to treat a *longitudinal section* of alcohol material with concentrated sulphuric acid. The walls of the sieve-tubes and the sieve-plates are dissolved. The slime-strings of old sieve-tubes, however, remain unaffected, and we can thus obtain preparations which show as in *D* in Fig. 47, and demonstrate in the clearest manner that the contents of the constituent cells join *one another through the pores of the sieve-plate*. These preparations can be washed by running water under one edge of the cover-glass, and withdrawing it from the opposite edge by means of blotting-paper; and can then be stained with a drop of aniline blne.

In order to obtain a thoroughly accurate idea as to the natural distribution of the contents of sieve-tubes, it is necessary to have recourse to another method of preparation, *viz.*, to fix the contents of the sieve-tubes of a pot plant of *Cucurbita* with boiling water, not, however, previously severing the stem, but bending it down under water for about five minutes. Material thus fixed can he either investigated direct, or can be preserved at will, and without further change, in alcohol. The contents of the sieve-tube will then be fixed in their position as during life, and it will be seen that the slime-strings and slime-plugs of the alcohol material are due to the method of preparation, and the plug no doubt to the stem having been cut before the action of the reagent. Such material will also show us that in active sieve-tubes the callus forms only a thin layer on the sieve-plate, while it thickens greatly in those which have ceased to function; *similarly, in the former case the sieve-pores are filled, not with* mucus, but with coagulated sieve-tube sap, while in the latter case the callus layer almost fills up the pores.

We may also prepare longitudinal sections of fresh material. In these the sieve-plates show just as clearly as in alcohol material. The accumulations of slime at the sieve-plates can be well seen; but the slime nowhere shows as a special string with-

drawn from the side-walls of the sieve-tube. The "slime-string," therefore, is due to the action of the alcohol. While these conditions hold good for the Cucurbitaceæ, active sieve-tubes show usually a non-coagulable cell-sap. In the lining layer of cytoplasm are found larger or smaller masses of slime, and small colourless leucoplasts, which usually produce very small starch grains rich in amylo-dextrin (*see* p. 124), and colouring red with iodine. In material examined in a fresh state we again find the contents of the tube collected unilaterally at the sieve-plate, and the starch grains accumulated there.

Thickening of Dicotyledonous Stems.—An extraordinarily favourable object for the study of the growth in thickness of Dicotyledons is the pipewort, *Aristolochia Sipho*, a hardy deciduous climbing plant not infrequently grown in England, and material for the investigation of which will therefore be probably not difficult to obtain. It is desirable to collect it at the end of June, using it then, or preserving it in alcohol. Our description will be made from fresh material, but, with the exception of the green colour, will serve also for alcohol material. First take a *cross-section* through a twig about $\frac{1}{8}$ or $\frac{1}{6}$ inch in thickness. This section (Fig. 48) shows with a lens an internal loose pith (*m*), around this a ring of separate vascular bundles (*fv*), farther outside a continuous white ring (*sk*), then green cortical tissue (*c*) (if alcohol material the green colour will be absent), and finally a yellowish-green peripheral rind (*cl*). With a low power under the microscope we can determine that the pith consists of large, round cells, in part filled with air. In the vascular bundle the wood (*vl*) appears darker, pierced by the large cavities of the vessels; then follows the cambial band (*fc*), composed of narrow, radially-

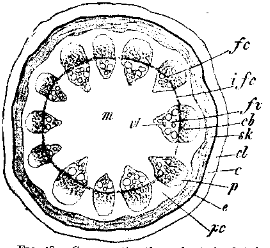


FIG. 48.—Cross-section through a twig of *Aristolochia Sipho*, about $\frac{1}{8}$ inch in thickness. *m*, pith; *fv*, vascular bundles, of which *vl* is the wood, and *cb* the bast portion; *fc*, fascicular cambium; *ifc*, inter-fascicular cambium; *p*, primary bast parenchyma on the outer side of the bast, which effects a transition to the ground-tissue; *pc*, pericycle; *sk*, sclerenchyma ring, belonging to the pericycle; *e*, starch sheath (endodermis); *c*, green cortex; *cl*, collenchyma (× 9).

arranged, bright cells; then the bast (*cb*), which appears somewhat less bright, and is also devoid of the regular arrangement shown by the cambial cells, and which is accompanied externally by a layer of brighter ground-tissue cells which are poor in cell-contents. In somewhat thicker sections, and with a low power, this layer is very distinguishable by its clearness, in contrast with the surrounding, somewhat chlorophyll-containing tissue. The white ring, next following outwards, is composed of strongly-thickened sclerenchymatous elements; between the vascular bundles it projects somewhat inwards. This sclerenchyma ring serves for the vascular system, as a whole, the same mechanical purpose as is served by the sclerenchyma sheaths of the individual bundles in *Ranunculus*. Next to this ring, and farther outwards, is a tissue containing chlorophyll, the innermost layer of which, bounding the sclerenchyma ring, is marked by its richness in starch, and belongs to the category of so-called **"starch sheaths"**. Such a starch sheath corresponds to the "endodermis" of roots, which we shall study hereafter, and forms the innermost layer of the primary cortex. The whole axial portion, enclosed within the starch sheath, is the **central cylinder** or **stele**; to it the sclerenchyma ring belongs, and to that peripheral portion of it, the **pericycle**, to which the parenchymatous tissue immediately outside the vascular bundles also belongs. After treatment with iodine, or chlorzinc iodine, the starch sheath stands out very clearly. To the inner chlorophyll-containing tissue of the primary cortex there follows a tissue, likewise to a small extent chlorophyll-containing, and with narrow cavities, with glistening cell-walls, more strongly thickened in the angles, which, by this last characteristic, we know to be **collenchyma**—a tissue generally appearing like a glistening white sheet pierced by rounded holes, and exceedingly common as a hypodermal tissue in Dicotyledons. Outside all is found the epidermis. The collenchyma ring is, however, interrupted by certain prolongations of the inner cortical parenchyma, rich in chlorophyll, which extend up to the stomata.

The Cambium Ring.—Having obtained this general information, we will now turn to our specific object, namely, to determine in what fashion the complete **cambium ring** of Dicotyledons is produced; and first we will examine a single bundle (Fig. 49) under a higher power. The *cross-section* must be sufficiently thin, and will be best prepared from alcohol material, and studied

either in glycerine or in chlorzinc iodine, the latter perhaps being preferred. The crushed protoxylem (*vlp*), and the vascular tracheïdes and vessels nearest to them, are enclosed in an abundant thin-walled xylem parenchyma (*p*). Farther outwards in

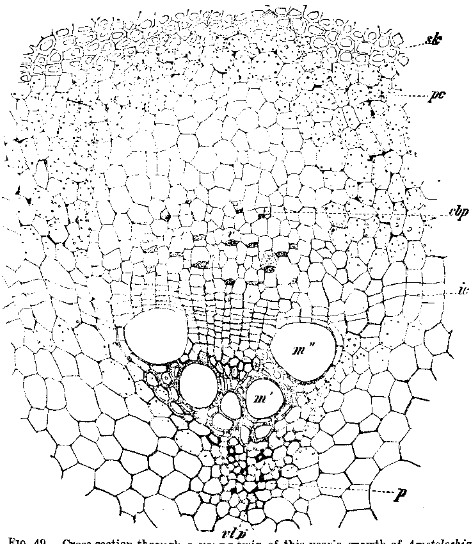


FIG. 49.—Cross-section through a young twig of this year's growth of *Aristolochia Sipho*, showing a vascular bundle after the cambium has commenced its activity. *p*, parenchymatous elements at the inner extremity of the wood; *vlp*, protoxylem elements; *m′* and *m″*, pitted ducts; *ic*, interfascicular cambium, continued into the fascicular cambium; *v*, sieve-tube; *cbp*, protophloëm elements; *pc*, tissue of the pericycle; *sk*, inner part of the riug of sclerenchyma-fibres (× 130).

the broadening vascular bundle, the vessels increase in diameter (*m′*), and have bordered pits. Between them the tissue is thickened and lignified; it consists of xylem parenchyma and narrow tracheïdes. The cambial-tissue which next succeeds shows thin-walled flat cells arranged in straight radial rows. This tissue is

in full activity, and the vessels nearest to it (m'') are manifestly still in course of development. In exactly the same way in the bast, or phloëm, the incomplete passes over into the completely-developed tissue, though here the transition is effected more rapidly. In the bast we can see the sieve-tubes (v) with their companion-cells, and between them parenchyma which we can call bast or phloëm parenchyma, in contradistinction to the xylem parenchyma. In the periphery of the bast we can see the **protophloëm** groups (*cbp*) enclosed in parenchyma, and consisting of narrow sieve-tubes and companion-cells. In the active sieve-tubes the companion-cells are found on their cambial side; they are distinguished by their small radial diameter and the richness of their contents. Phloëm parenchyma is wanting in the bast of Monocotyledons, but is well-nigh universal in Dicotyledons, so that the case of *Ranunculus* in which it is not present must be looked upon as exceptional in this respect.

If the cross-section has been taken from a twig in a proper stage of development, we see between the cambium of the separate vascular bundles (the **fascicular cambium**) the commencing formation of the **interfascicular cambium** (*ic*). In the elements of the ground-tissue, the conjunctive parenchyma, which are interposed between the vascular bundles, and constitute the primary medullary rays, we can see the interpolation of tangential division walls, so that streaks of cambium are formed which connect the cambiums of the circularly-arranged vascular bundles into a closed **cambium ring.** As the accompanying figure (*Fig.* 49) shows, the method of formation of this interfascicular cambium is in this case extraordinarily easy to follow; and the original contour of the ground-tissue cells which have undergone division can be long recognised. The cambium ring, by means of which Dicotyledons and Gymnosperms in general thicken, is formed therefore of an alternation of fascicular and interfascicular portions. The **secondary tissues** developed by the activity of this cambium ring and upon its inner side are wood or xylem tissues, those developed on its outer side are bast or phloëm tissues. A sharp limit between the primary and secondary tissues is not distinguishable so far as the original bundle is concerned; but the interfascicular bundles, naturally, are wholly secondary.—The progress of lignifaction after cambial development can be very beautifully followed by the phloroglucin and hydrochloric acid method (*see* Chap. V., p. 78).

The actual nature of most of the elements constituting the vascular bundle can only be realised in longitudinal sections, though their distribution is best seen in the cross-section. A delicate *radial-longitudinal section*, which has cut through pretty near the middle of a vascular bundle, shows in that portion of the bundle nearest the pith, elongated primary wood parenchyma, with horizontal end walls; between them very narrow annular vessels, or vascular tracheïdes, more or less crushed, then somewhat broader annular vessels, perhaps partially showing transitions to the spiral form; then closely wound, still broader, spiral vessels, partly showing transitions to the reticulated form; and finally the broad pitted ducts, with bordered pits. Between these various forms of vessel or tracheïde can be seen very elongated tracheïdes with tapering ends, devoid of contents, and also with bordered pits; and in thick-walled wood parenchyma, short, with horizontal end walls, and broad unbordered pits, and usually containing starch. The immature **ducts** appear as broad, cylindrical thin-walled cells, separated by horizontal end walls, with a thick lining layer of cytoplasm, and with nucleus. In older ducts the end walls are swollen, and the contents reduced. In the fully-formed duct these contents are no longer observable, and the end walls are reduced to a narrow ridge projecting inwards as a thickening ring. The flat tabular cells of the **cambium** ring show abundant cytoplasmic contents nuclei, and delicate partition walls. The sieve-plates of the **sieve-tubes** are oblique, and present to the observer their surface with darker shining dots. The side walls of the sieve-tubes are likewise provided with small, finely-punctate sieve-pits, usually elongated obliquely. In the periphery of the bast the **callus plates** are already formed, covering the sieve-plates with a highly refractive layer. The small sieve-pits on the side walls also have small callus plates. By the sieve-tubes will be noticed the companion-cells, densely filled with cell-contents, and the broader, shorter cells of the bast parenchyma, usually with starch-contents, but less protoplasm. The bast is separated from the ring of sclerenchymatous elements by broader somewhat more elongated parenchyma cells of the ground-tissue. The sclerenchyma-fibres of the ring are very long, pointed at their ends, with their ends interpolated between one another, and are provided with fine pits. The elements of the starch sheath are relatively short; then follow the rounded cells of the chlorophyll-

containing inner cortex. Then comes the collenchyma ring, and we can determine that the elements composing it are many times as long as broad, and join on to one another with oblique end walls.

It will have been noticed that *Aristolochia* has no vascular bundle sheath of sclerenchymatous tissue surrounding the individual bundles, as was found in *Zea* and also in *Ranunculus*. The purpose of these is here subserved by the pericyclic sclerenchyma ring, surrounding the vascular bundle system as a whole, in the form of a closed woody cylinder. This cylinder is ruptured later on by the progressive growth of the vascular bundle ring.

If we have an opportunity of examining *cross-sections* of an older stem of *Aristolochia* it may not be amiss to get in it a general idea of the results of cambial action and the other changes which have taken place. We shall see that the pith is partially destroyed, and instead of preserving its cylindrical form, as it generally does, has in this case become flattened more or less, perhaps into a mere streak. Through the activity of the cambium the primary vascular bundles have increased considerably in size, and the interfascicular cambium has formed between them secondary bundles, which make up the woody ring. The medullary rays are narrow, and more numerous, the cambium adding to their length proportionally to the growth of the bundles. The cells of the medullary rays are radially elongated. If the flattening of the pith has proceeded to a great extent, nearly all of the medullary rays will appear more or less curved; at one end, leaving the pith at right angles, while the other, if prolonged, would end at right angles to the epidermis. The cambium layer is very recognisable, and the bast will be seen to have also increased in quantity. The sclerenchyma layer may no longer be continuous, but broken up, by the increase of the inner tissues, into isolated greenish-looking strands. We will not, however, further concern ourselves with the structural features of *Aristolochia*, as we propose to study the subsequent thickening of stems hereafter in other material.

If the stem of *Aristolochia Sipho* is not available, almost equally good material can be found in the young seedling stems of *Ricinus communis*, which can be grown for the purpose; as also can the Sunflower (*Helianthus annuus*), or the Jerusalem Artichoke (*H. tuberosus*), the latter being grown from seed or tubers. The deviations from the structure detailed above will offer no difficulties.

CHAPTER X.

STRUCTURE OF THE CONIFEROUS STEM.

PRINCIPAL MATERIALS USED.

Twigs of *Pinus sylvestris*, or other *Pinus*, not more than ½ inch in thickness; fresh.

Pieces from a thick stem of the same, cut in June or July; preserved in alcohol, and laid for two or three days before use in half-and-half glycerine and alcohol.

PRINCIPAL REAGENTS USED.

Chlorzinc iodine—Alkanet root—Böhmer's logwood, or hæm-alum—Aniline blue—Iodine glycerine.

Pinus sylvestris.—We will now take the Scotch Fir (*Pinus sylvestris*), and make a careful study of the structure of the stem. It is characteristic of the Pinaceæ that the secondary growth of the wood consists almost wholly of but one kind of element, typical **tracheïdes**, between which in a certain number of species only, as, *e.g.*, the Scotch Fir, strings of wood parenchyma are intercalated. True tracheæ are wanting in the Pinaceæ, and spirally-thickened vessels, or vascular tracheïdes, are found only in the medullary sheath, in the protoxylem of the vascular bundle. Even in stems of half an inch in thickness, we can find these very easily. In cross-sections passing through the pith (which is readily indicated to the naked eye by its darker colour) it can be seen that the inner limits of the ligneous mass consist of portions projecting into the pith and composed of elements with narrow cavities, and with somewhat brownish walls. In delicate radial longitudinal sections passing through the same region we can determine that these elements are spiral vessels or vascular tracheïdes. Some such vessels, which possess at the

same time spiral bands and bordered pits, serve as a transition to *the typical tracheïdes having bordered pits only.*

As to the directions of the sections which we shall need, as well as the distribution of the tissues which we shall study, we can first of all get a general idea by means of the lens, or the low power of our microscope used as a lens, using for the purpose a piece of fresh stem not more than half an inch in thickness. The distinction of wood and bast, the annual rings, resin passages and medullary rays will then be distiuguishable (*see Fig. 50*).

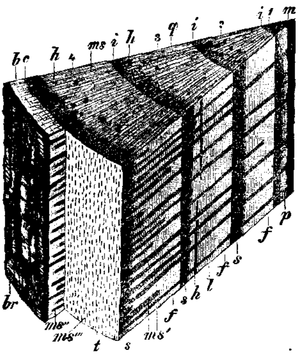


FIG. 50.—*Piece of a four-year-old stem of the Scotch* Fir (*Pinus sylvestris*) cut in winter. *q*, appearance in cross-section; *l*, in radial longitudinal section; *t*, in tangential longitudinal section; *f*, spring wood; *s*, autumn or late summer wood; *m*, pith; *p*, primary wood; *1*, *2*, *3*, and *4*, the four successive years rings of the wood; *i*, *i*, limits of the annual growths; *ms*, medullary rays as seen in cross sections in the wood; *ms'*, in longitudinal section; *ms''*, as seen inside the bast; *ms'''*. as seen in tangential longitudinal sections; *c*, cambium ring; *b*, bast zone; *h*, *h*, resin passages; *br*, the primitive bark (× 6).

In our careful study we will use a high power, and as we shall also take cognisance of the contents of the elements, and especially concern ourselves with the cambium, we think it preferable to use material which has been preserved in alcohol. Fresh wood tears at the cambium, and dry wood does not differentiate so well. Alcohol has the further *double advantage*, that it will have dissolved the abundant resin out of the wood, and will have fixed the cell-conteuts. It is desirable that the pieces of stem which are to be preserved in alcohol shall be moderately thick, since the size of the elements constituting the wood of a Conifer is progressively greater up to a certain age. In order to fix the cambium in an active condition the material is best collected in June or early in July, *i.e.*, at the time when the cambium is most active. The material must include the periphery of the stem, but on the other hand need

not extend deeply into the woody mass, *i.e.*, the central portion of the stem can be discarded.

In order that the alcohol material should not become dry, and the elements filled with air, while being cut, it is recommended to lay the pieces required for study in a mixture of equal parts glycerine and alcohol for at least twenty-four hours before they are needed. Material prepared in this way is also much easier to cut, as the hardness which alcohol gives is in great measure rectified. The sections are then examined in glycerine.

Sections prepared from a stem must be quite accurately directed. We shall need them in three different directions (*cf.*, Fig. 50); the *cross-section,* which must be exactly at right angles to the long axis of the stem, the *radial longitudinal section,* which must follow accurately the direction of the medullary rays and the *tangential longitudinal section,* which must be cut perpendicularly to the medullary rays. This latter will, of course, only be tangential in a limited sense, and the strictly tangential part of the section must be sought for; also the thicker the stem the better this section may be. The cross-section must include wood, cambium and bast, and similarly with the radial longitudinal section. Tangential longitudinal sections must be taken from at least two parts, one in the wood and the other in the bast

In the preparation of these sections the quality and condition of the razor becomes of prime importance. If it be at all blunt the inner thickening layer will be torn from the tracheïdes, and the section will be useless. Small sections will suffice, but they must be as thin as they can possibly be made; and all the precautionary measures already noted must be taken to avoid injury to the razor. If the razor is very hollow ground, correctly directed sections can only be taken from a narrow piece of material, or near the edges of a broader piece; for only so long, *i.e.*, as the back of the razor does not rest on the cut surface. Hence only slightly hollowed razors should be used for wood sections; and it would be indeed much preferable to have for this purpose a razor which is ground flat upon one side only (the under one in cutting), but that these razors are difficult to sharpen properly. The surface of the material should be prepared for the razor with a sharp pocket-knife or scalpel, or an old thin-bladed razor may be kept (sharp) for this purpose. Under any circumstances the first sections made should be rejected, as they will include the tissues torn in preparation (*see* Chap. V.).

A *cross-section* extending through wood, cambium and bast, shows us the **tracheïdes** arranged in very definite radial rows. As we trace such rows outwards here and there they are seen to double, or fork, to accommodate themselves to the increased periphery of the stem. In section the tracheïdes appear mostly four-sided, now and then five- or six-sided. As we pass along a row outwards, they are seen to become progressively narrower in their radial diameter, and their walls more thickened, up to a point at which the cavity is quite flat; then abruptly, without transition (Fig. 53, on p. 144 at *g*), we note a very large tracheïde, followed by another and another until the progressive diminution sets in again. In each radial row the abrupt change in dimension takes place, so that a clearly distinguishable curved line can be traced in the section. This line marks the limits of a year's growth, and is visible to the naked eye upon a section of a stem or on the end of a plank of pine-wood. During the summer the tracheïdes which are successively formed by the activity of the cambium are more and more flattened and thicker walled—the most flattened and thickest walled being those which are last formed in a season's growth; while the tracheïdes which are formed when growth is resumed in the ensuing spring are of the largest size, and have the thinnest walls. We can distinguish in this way between wide-cavitied spring wood and narrow-cavitied summer wood, the line of demarcation representing the position of the cambium during a winter's rest. Hence, if we were to take a section of a twig cut off during winter, the wood in contact with the cambium, on its inner side, would be composed of the narrowest cavitied tracheïdes (such a section is shown in the subjoined Fig. 51); and a fairly shrewd judgment as to the time when material is cut for preservation could be made by comparing the tracheïdes adjoining the cambium with those found in the annual rings next within. Interpolated amongst the elements may be found occasional small cavities, representing sections through the tapering ends of tracheïdes. Parallel to these radial rows of tracheïdes run similar radial rows of narrow elements, elongated radially, sometimes containing starch and protoplasm, sometimes only water, the rows usually only one cell broad but occasionally more. These are the **medullary rays.** **On the radial walls of the tracheïdes, *i.e.*, the walls parallel with the radii, and therefore facing the medullary rays, we shall see the remarkable and characteristic bordered pits. If the section**

be only moderately thin, these will look like narrow spindle-shaped or lenticular hollows in the thickness of the wall; *but if very thin they appear like two pairs of open pliers directed towards one another, each pair enclosing a narrow lancet-like arch* (*cf.* Fig. 26, *C*). The pit is crossed from apex to apex of the two arches, by the delicate **closing membrane,** which thickens in the middle into the **torus**, or, to be more accurate, the two pit chambers are separated from one another by this closing membrane, which includes a continuation of the middle lamella of the tracheïdes (*see* Fig. 27); while the small gap between the corresponding ends of the jaws of the opposing pairs of pliers indicates the opening of the bordered pit into the cavity of the tracheïde. The pit, therefore, broadens rapidly as we approach the closing membrane, and it is this broadening, so as to produce a hollow or chamber in the thickness of the wall, which distinguishes a bordered (or chambered) pit from a simple pit such as we saw in *Ornithogalum*, in the Date, and in the cortex of the Cherry laurel (*Prunus Lauro-Cerasus*) as described on p. 73. Upon the tangential walls of the tracheïdes of *Pinus sylvestris* bordered pits are rarely found, though frequent in the autumn tracheïdes of allied trees. In the later tracheïdes of the year the bordered pits are much smaller. Between the tracheïdes and the cells of the medullary rays, so far as the latter have protoplasmic contents, are very broad, unilaterally bordered pits—pits so broad that they occupy almost the entire width of the tracheïde. The border is developed only upon the wall of the tracheïde, not upon that of the medullary ray cell. **The closing membrane bulges into the tracheïde; it has no torus. Between the tracheïdes and those medullary ray cells which con-**

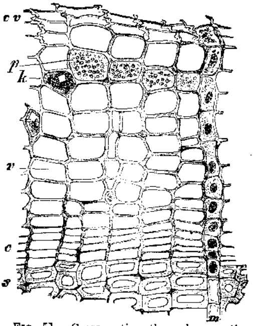


FIG. 51.—Cross-section through a portion of the stem of *Pinus sylvestris* taken during winter, with the cambium at rest, and passing through autumn wood, cambium and the adjoining bast. *s*, autumn wood: *c*, cambium; *v*, sieve-tubes; *p*, bast parenchyma; *k*, crystallogenous cell of bast; *cv*, sieve-tubes which has ceased to function, and have collapsed; *m*, medullary ray.

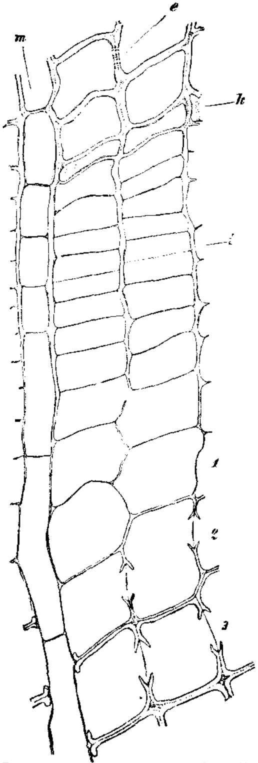


FIG. 52.—Part of a cross-section of a pretty old branch of *Pinus sylvestris*. The strip passes through the cambium (*i*, initial layer), and ends on the one side in the young wood, on the other side in the young bast. *1*, *2*, *3*, stages in the development of bordered pits; *m*, medullary ray; *e*, sieve-plate; *k*, flattened cells with brown contents, later on containing crystals (× 540).

tain only water, the pits are bilaterally bordered, just as between tracheïde and tracheïde.

In the immediate neighbourhood of the cambium we see the young wood composed of incompletely developed tracheïdes (Fig 52). The walls of the cells diminish rapidly in thickness towards the cambial zone. Inside the cambial zone the radial walls thicken again. What we have called **Cambium** consists of an **Initial layer** of cells (Fig. 52, *i*) which, through continued tangential divisions, gives off tissue mother-cells towards wood and bast, from which the elements of the wood and the bast have their origin. Upon the wood side the successive stages in the development of the bordered pits can be followed (Fig. 52, *1*, *2* and *3*). The radial rows in which the tracheïdes are formed can be traced out through the cambium into similar radial rows of bast elements, for these show every whit as marked a radial arrangement. On the bast side of the cambium the cell-walls rapidly thicken, and take on a dull-white appearance. On the radial walls of the wide-cavitied elements of the bast (which

are **sieve-tubes**) corresponding to the places where, in the wood, bordered pits stand, sieve-pits (*e*) will be found; in very delicate sections we can recognise the fine pores which traverse these sieve-pits. Companion-cells are not developed to accompany the sieve-tubes of Gymnosperms. Unilamellar bands of bast parenchyma alternate with the thicker layers of sieve-tubes. They are in the main composed of starch-containing cells, which in the older bast swell considerably. Between the starch-containing cells, crystallogenous cells are scattered, which are very early marked by their brown colour (*k* in Figs. 51 and 52). Only a proportionately thin zone of the bast is composed of turgescent elements retaining their original order; on the outer side of this zone the radial rows become crumpled (*see* Fig. 51 *cv, ante*), the cell-walls begin to turn brown, the cell-cavities are more or less crushed. Only the starch-containing cells of the bast and the medullary ray cells enlarge to any extent; these become rounded, and appear now as more or less spherical elements, densely filled with starch. Ultimately the sieve-tubes and crystallogenous cells are quite crushed, become tangentially stretched, and separate, in the form of lamellæ, the successive layers of large starch-containing cells. The outer part of the bast appears, therefore, to consist of these last alone. Externally we note thin layers of cork, and the deep brown dead tissues, the **Bark**, which these have peripherally cut off.

Resin Passages.—Thus far we have left unnoted the patches of wood parenchyma which every cross-section of the wood shows, and which always enclose a **resin passage** or **resin canal** (Fig. 53). In alcohol preparations this canal will have lost its resin contents. The cross-section of the wood cuts the resin canals also in cross-section; each of these presents the appearance of an intercellular passage (*i*), which is surrounded by a layer of larger, thin-walled cells, the **epithelial cells.** The walls of these cells are brownish; they contain a large nucleus and a lining layer of protoplasm. To these cells adjoin as a rule starch-containing wood parenchyma cells (*a*), sometimes also a medullary ray. Their method of development shows that these resin passages arise *schizogenously*, that is, by the separation of cells originally in union. The resin passages which we have cut in this way in cross-section run vertically through the wood, but are connected together by horizontal ones, which lie within broad medullary rays, and which in the cross-section are occasionally exposed in

longitudinal view. We shall see these connecting resin passages again in the tangential longitudinal sections.

A *cross-section* through *fresh* pine-wood (Fig. 53) shows that the resin canals are filled with resin. This appears in the preparation in the form of strongly refractive viscid drops of often irregular contour. If we run in a little alcohol, the resin drops quickly disappear. We can, moreover, stain them in a characteristic fashion with the red colouring material of alkanet roots (roots of *Alcanna tinctoria*), which we have already used to colour oils. For this purpose we take a cross-section through the wood of the Scotch fir, and lay it on the object-slide in a drop of water. We then take a similar thin section from the bark of a dried alkanet root, blow off from it any loose particles, lay it upon the fir section, and cover with a cover-glass. Then run in a drop of about 50 per cent. alcohol under the cover-glass, and allow the object to stand for from half an hour to an hour. If now the alkanet bark is removed and the section of fir examined, the parts containing resin appear stained a beautiful dark-red colour, while the other parts of the preparation remain entirely uncoloured.

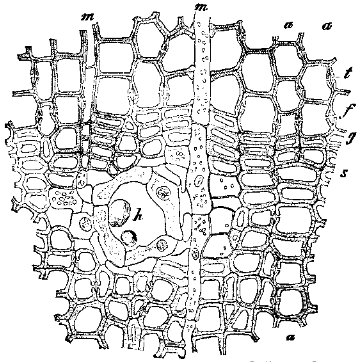


FIG. 53.—Part of a cross-section through the wood of the Scotch fir at the limits of a year's ring. *f*, spring wood; *s*, late wood; *t*, bordered pit; *a*, a radial row of tracheïdes which doubles; *h*, resin passage; *m*, medullary rays (× 240).

Colour Reactions.—*Cross-sections*, preferably of alcohol material, if treated with chlorzinc iodine show the walls of the tracheïdes yellow-brown; their innermost thickening layer, immediately surrounding the cell-cavity, is, however, here and there stained violet. In the neighbourhood of the cambium, in tracheïdes which are not yet fully formed, the protoplasmic contents and

nucleus are now easy to see. It can be settled with equal certainty that the tracheïdes, when fully formed, lose all living contents. The walls of the cambium, and the youngest adjoining cells, have stained bright violet, those of the older portions of the bast a dark violet colour.[1] The contents of the crystallogenous cells remain brown, while those of the cells of the cork-layer now appear reddish-brown. The specially thin inner walls of the cells surrounding the resin-canal, *i.e.*, those actually forming the resin-passage, usually stain a dirty violet. Careful examination shows, moreover, that the closing membrane of the unilaterally-bordered pits has stained violet, while that of the bilaterally-bordered pits remains uncoloured. Starch, wherever present, takes the iodine reaction.

In Chap. V., p. 78, we have already seen the **Lignin** reactions displayed by coniferous wood. Corallin also, in virtue of properties already known to us, stains the lignified cells quite differently from those which are unlignified. We obtain, indeed, very beautiful and instructive preparations when the sections are laid for some time in soda-corallin and then examined in glycerine. The lignified membranes are stained a deep red; towards the cambium this red disappears, and passes over into pale yellow. In the bast the cell-walls have a pale reddish-yellow coloration; the sieve-plates are coloured deep red, especially where they are covered with a callus-layer. As corallin also stains starch grains rose-colour, these consequently stand out quite sharply in the outer parts of the bast.

We will now prepare some *radial longitudinal sections*, again using the alcohol material.

Structure of the Wood.—Such a section shows us in the wood the **tracheïdes** with **bordered pits.** The ends of the tracheïdes interlock. In the spring tracheïdes these ends are rounded; in the late summer tracheïdes they are pointed. The bordered pits in face view show the form of two concentric circles (Fig. 27 *A*). The inner small circle, or (occasionally) ellipse, indicates the opening of the pit into the cavity of the tracheïde; the large outer circle indicates the broadest part of the pit, that which is bounded by the primitive wall separating the two tracheïdes. It is this primitive wall which, thickened at its middle into the **torus,** constitutes the closing membrane. The torus can often be distinguished as a faintly shining disk of about twice the diameter of the mouth of the pit. Especially in sections of dry

[1] See note on page 152.

wood the closing membrane round the torus sometimes shows radial striation (Fig. 27 *A*). The torus can be shown up clearly *if the section is laid for about fifteen minutes in Böhmer's logwood solution, or in hæm-alum*; but sections taken from alcohol material must previously lie for a little while in water in order that no precipitate of logwood be formed on the surface. In the narrow late summer tracheïdes the bordered pits are small and less numerous.[1]

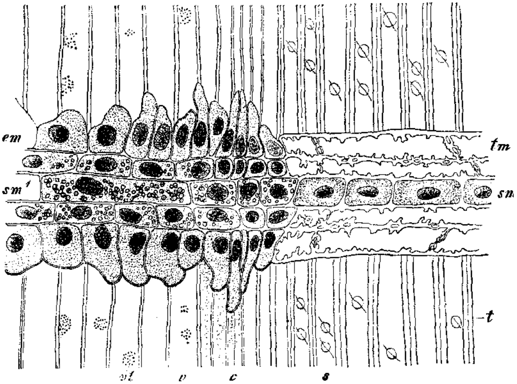


FIG. 54.—Radial longitudinal section through the stem of the Scotch fir, at the outer limits of the xylem, through the cambium, and the adjoining bast, and showing also a medullary ray. *s*, autumnal tracheïde; *t*, bordered pit; *c*, cambium; *v*, sieve-tube; *vt*, sieve-area; *tm*, tracheïdal medullary-ray cell; *sm*, starch-containing medullary-ray cell in the xylem; *sm*¹, in the phloëm; *em*, proteid-containing medullary-ray cell (× 240).

Traversing the tracheïdes horizontally we see the cells of the **medullary rays** (Fig. 54, *sm*), the structure of which we can study more readily now than in the cross-sections. The medullary rays usually have but little height, *i.e.*, in the direction of the length of the tracheïdes and therefore of the length of the

[1] It would be well for the student also to refer back to the section on "Bordered pits of *Pinus*" in Chap. V., as some further details are to be found there.—[ED.]

stem; they are found, however, up to so many as sixteen cells high. They consist of radially-elongated cells, lying side by side in rows. The median cells contain protoplasmic contents, and usually also starch, and show on the sides towards the tracheïdes the large shallow unilaterally-bordered pits already noted; the upper and under rows of cells, from one to three in number, have no protoplasmic contents, contain under natural conditions only water, and are connected with the tracheïdes by means of small bilaterally-bordered pits, and also show peculiar jagged or serrate projecting ridges on the walls directed tangentially, which may protect against crushing. Similar rows of cells may also be interpolated in the median parts of very deep medullary rays; while there are medullary rays which consist wholly of them, and others which contain none. From their pits and want of living cell-contents these rows of cells resemble, in structure and relations, the tracheïdes of the wood, and on these grounds might be considered to be tracheïdal elements of the medullary ray. These tracheïdal elements of the medullary ray probably facilitate water diffusion in a radial direction between the tracheïdes of the wood; while the elements which have protoplasmic contents carry reserve food-stuffs in the same directions.—The radial longitudinal section may also have cut through a string of secondary wood-parenchyma, and have laid bare its **resin-canal.** The parenchymatous cells surrounding the resin-canal are convex towards it, and about as broad as long; those more external are clearly longer. In a larger medullary ray we can perhaps trace the course of a horizontal resin-canal, and possibly see that ultimately the vertical and horizontal resin-canals intercommunicate.

The **cambium** in longitudinal section shows on the one hand as narrow elongated cells, with end-walls more or less inclined, in which are protoplasmic contents and nucleus, and from which the elements of the wood and bast proceed; and, on the other, as shorter, broader, but otherwise similar cells, which are continuous on both sides into the cells of the medullary rays.

The Bast.—In the bast we will specially study the **sieve-tubes** with their **sieve-areas**; and in order to be able to see these latter well and readily, we lay some *radial longitudinal sections*, which we have prepared from alcohol material, in a watery solution of aniline blue. This stain has, like corallin, the property of deeply staining the **callus** of sieve-tubes, *i.e.*, the substance

callose. The sections need remain only a few minutes in the stain, and can then be transferred to glycerine. This latter allows the colour to remain in the sieve-plates only, removing it from all other parts of the section. The sieve-plates cannot now be overlooked in microscopical examination. Their colour is a beautiful blue, and so permanent that the preparations can be preserved. *We can distinguish the sieve-plates even in the immediate neighbourhood of the cambium, and follow them into the parts in which the sieve-tubes have become crushed, and the sieve-plates have therefore lost their radial position.* Before this, however, the sieve-plates will have lost their callus-layer, and with it their capacity for staining. The sieve-tubes have the form of the cambium cells from which they have developed; they bear sieve-plates only on their radial walls, just as tracheïdes have bordered pits. They present the appearance of round or oval spots, which are collected into an indefinite number of somewhat angular, finely-punctate areas (Fig. 55). At some distance from the cambium the sieve-areas are covered by a homogeneous shining substance, staining sky-blue in the aniline blue —the **callus-plate.** Further away still this is again dissolved, the sieve-area is bare, and no longer stains; the sieve-tubes are now functionless. It is not difficult to see that the active sieve-tubes contain a thin cytoplasmic lining layer, but as a rule no nucleus, this having disappeared during the development of the tube. They contain also watery albuminous solution, grains which colour yellow with iodine, and probably are leucoplasts, and likewise grains and flocculent masses which give with iodine the port-wine red amylo-dextrin reaction, and probably represent an intermediate product in the transformation of starch into dextrin and maltose. *These contents point to the sieve-tubes playing a very important part in the nutritive processes in the plant.*

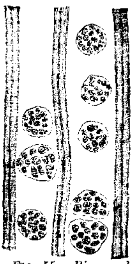


FIG. 55.—*Pinus sylvestris.* Parts of two sieve tubes with sieve-areas (× 540).

The **crystallogenous cells** *of the bast are distinguished in longitudinal section by their brown contents;* they are comparatively short, and commonly with horizontal walls, and have apparently arisen from horizontal division of cambial cells. Th[ey] contain numerous prismatic crystals lying upon and near o[f]

another. The starch-containing cells likewise stand out clearly. They are still shorter than the crystallogenous cells, lie in rows one above the other, and are also interpolated singly or in longer rows between the crystallogenous cells. Later, these starch-containing cells swell very considerably.

A similar section of fresh material, treated with concentrated solution of molybdate of ammonia in saturated ammonium chloride solution, shows a **tannin** precipitate in the crystallogenous cells. Similarly if a piece of stem be placed for two or three days in a concentrated aqueous solution of potassium bichromate, and sections afterwards taken, a brown precipitate is found in the cells.

The **medullary rays** can be easily traced from the wood into the bast, and in doing so undergo a characteristic change. In order readily to appreciate this we lay some of our radial longitudinal sections in iodine glycerine, and examine them in it. Those cell-rows which in the xylem are tracheïdally developed, can be for the most part traced into the bast as protein-containing cells. These albuminous cell-rows of the medullary rays are distinguished by greater height and smaller length, and are closely applied to the sieve-tubes, with which they are connected by sieve-plates. In that portion of the bast in which the sieve-tubes have become functionless, these albuminous medullary-ray cells have also lost their contents, and become crushed. From this we infer that these protein-containing medullary-ray cells have in the Scotch fir the function of companion-cells. These albuminous medullary-ray cells contain no starch, while the starch-containing, and also some of the tracheïdal, medullary-ray cells of the wood can be traced into starch-containing rows of medullary-ray cells of the bast. Protein-containing cell-rows in the medullary rays are not found in the bast of all the Coniferæ; in their place protein-containing rows of cells may be present in the bast parenchyma. We may also note that in the Scotch fir these albuminous medullary-ray cells first appear in the secondary growth, and are not found in the primary bundles.

The *tangential longitudinal sections*, which we likewise prepare from alcohol material, must be taken through at least two positions, namely in the wood and in the bast.

The **wood** section shows us the tracheïdes with unilaterally tapering ends. The bordered pits which have been cut through

appear exactly as in the cross-section (Fig. 27 *B,t*), and good sections of them are proportionally more easy to obtain than in the latter case. In old tracheïdes we can determine that the closing membrane bulges to one side, and that the torus (*t*) is pressed up against one of the openings of the bordered pits. Staining the section with hæmatoxylin will facilitate the proof of this. The sections of the medullary rays (Fig. 56) appear to have a spindle-like outline (*tm*, *sm*) since the cells at both edges (or ends) gradually become narrower. The shallowest medullary rays are about three cells high; most are about eight cells, while with some the height can increase up to as many as twenty cells. The shallowest are always but one cell broad; the highest can in their central part be several layers thick, and these latter contain the resin-canal, which is shown in section. The section may have also cut through a vertical resin-canal, which will present the same appearance as in the radial longitudinal section.

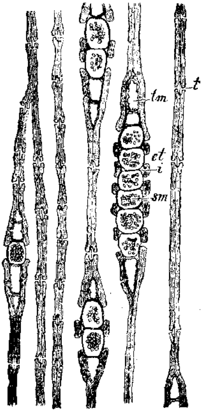


FIG. 56.—Tangential longitudinal section through the autumnal wood of the Scotch fir. *t*, bordered pit; *tm*, tracheïdal and *sm*, starch-containing medullary-ray cell; *et*, unilaterally bordered pit; *i*, intercellular space in the medullary ray. (× 240).

In this tangential section the tracheïdal elements of the medullary ray (Fig. 56, *tm*) can, without difficulty, be distinguished from those which contain protoplasm; on the other hand careful observation and a very thin section are required in order to determine that the medullary ray is accompanied on both sides by small intercellular spaces (*i*), which follow it along its course. These accompany almost exclusively the medullary-ray cells which are provided with plasmic contents, not the tracheïdal cells. Through these interstices, which can be traced from the periphery of the stem through the cambium right into the woody mass, the gaseous interchange with the surround[illegible] atmosphere which is necessary for the living cells is carried In sections through fresh material these intercellular [illegible]

are filled with air, and on that account appear dark and are easier to see. In alcohol material the alcohol has removed the air.

A section of the **bast** which will answer our requirements *cannot be obtained without trouble. There is no recourse but to* commence with the older and outer part of the bast, and prepare a considerable number of successive sections, till we have reached the young wood. We scan these sections with a low power objective, and look for such as contain still active **sieve-tubes**. By this means we can learn something about the callus-plate, which at once attracts the eye, even without staining and with slight magnification, as a strongly refractive pad (Fig. 57, *B*) attached to the cell-wall. The structure of these plates, with their sieve-pits can be best studied in these sections with the aid of aniline blue, as explained *on* pp. 147, 148. The *form* of the sieve-pit is the same from this point of view as in the cross-section; but the number which the section will have exposed is very large, and there is, therefore, greater probability of finding one thin enough for study. They can be found most readily at the edges of the section. The pits are seen in profile (Fig. 57), inside the radial walls of those sieve-tubes which the razor has cut. They are traversed by groups of very fine threads, which have been deeply stained. The callus plates (*B*) appear conspicuously blue. Their size first of all increases; they disappear from the sieve-tubes which have lost their function, just as do the callus threads which traverse the sieve-pits.

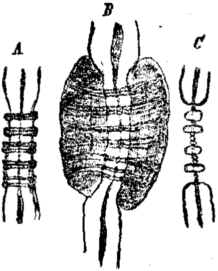


FIG. 57.—*Pinus sylvestris.* Portions of the wall of sieve-tubes after staining with aniline blue. *A*, Prior to the formation of the callus plate; *B*, after its formation; *C*, from a sieve-tube which is past its period of activity *and become functionless* (× 1500).

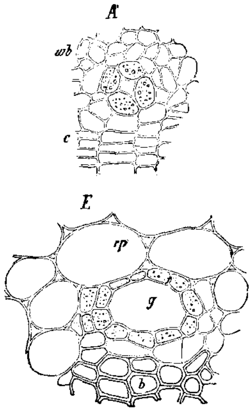


Fig 57*.—Resin-passages in the bast of a young stem of *Hedera helix* in transverse section (× 800). *A*, early stage; *E*, later stage; *g*, the resin-passage surrounded by its secreting cells; *c*, the cambium layer; *wb*, soft bast; *b*, bast fibres; *rp*, cortical parenchyma. (After Sachs.)

The **resin-canals** which we have here met with, and which in the cortical parenchyma of Conifers often attain very considerable size, can have their origin well studied in the Ivy (*Hedera helix*). Cross-sections of young stems will show the resin-canals in the central pith as well as *outside the xylem ring*, even in quite close proximity to the cambium ring. In the same section they will be met with in different stages of development, from a group of four cells, having between them a barely recognisable intercellular space, to fifteen or twenty cells surrounding a large cavity. The cells are at once recognisable by their granular contents. The mode of origin of the resin-passage is easily observable. It arises through the separation of the secreting cells, while these cells divide radially, and more rarely tangentially (Fig. 57* *A*), and thus surround a cavity continually enlarging up to a certain point. *The resin-passage, therefore, as in Pinus, is* **schizogenous** (compare Fig. 57*).

NOTE ON COLOUR REACTIONS IN THE CAMBIUM (p. 144-5).

The use of chlorzinc iodine on cross sections of alcohol material taken *through the cambium layer is very instructive.* The radial walls in the cambium itself show the bright violet coloration which is characteristic of cellulose walls generally; but the newly formed parallel division walls (tangential) appear *stained* yellowish. *This is because they are composed of pectic substances,* which will only later on become thickened by cellulose wall-layers (see also p. 64).

CHAPTER XI.

STRUCTURE OF THE STEM OF THE LIME—STUDY OF TISSUE-ELEMENTS BY THE MACERATION-METHOD—TYLOSES, OR TRACHEAL PLUGS.

Principal Materials Used.

Twigs of *Tilia europæa*, three or four years old; fresh.

The same, outer parts of a thick stem; in alcohol, and softened for a few days in half-and-half alcohol and glycerine.

External parts of ten to twelve year old branches of *Robinia Pseud-Acacia*; in alcohol; softened in glycerine-alcohol.

Principal Reagents Used.

Iodine-glycerine—Chlorzinc iodine—Corallin—Chlorate of potash and nitric acid.

Stem of Tilia europæa.—As a further object of study, we will take the Lime tree (*Tilia parvifolia*, or other form of the aggregate species, known under the name of *Tilia europæa*). A *cross-section* through a twig about one-fifth of an inch in thickness shows us a large-celled **pith**, the air-containing cells of which are grouped, rosette-like, around individual narrower cells, filled with finely granular brown contents. In the outer part of the pith lie **mucilage sacs**, forming hollows in the parenchymatous tissue, but which, however, are already empty. At its outermost limits the pith consists of smaller cells, filled with finely granular contents. Into this small-celled tissue project the **protoxylem** elements of the vascular bundles. The "unwinding" spiral vessels (from which the spiral thickening thread can be withdrawn), are noticeable in the cross-section, from the thickening bands coming out here and there. We can count about five **annual rings** in the cross-section of a twig of one-fifth inch thickness, and we shall perhaps notice that the successive annual rings can vary greatly in thickness. In each year's ring large

vessels (ducts) are first produced in close succession, and this it is which especially marks out the limits of the annual growth. Later on in the year's growth the broad ducts either arise singly or in isolated groups; in the last phase of the year's vegetation the cambium produces only elements with narrow cavity. Outside the cambium we see the keel-shaped masses of the **bast**, thinning off outwards. In these can be seen an alternation of tangentially-arranged bright and darker layers. The shining white layers are composed of numerous closely-united **bast fibres**, the walls of which are thickened almost to the disappearance of the cavity, the cavity of each element showing only as a dark dot. The layers have an irregular outline, and may be interrnpted here and there. The darker layers between the white consist of (1) narrow-cavitied starch-containing bast parenchyma cells, which are especially in contact with the bast fibres, and of (2) wide-cavitied elements, sieve-tubes, with companion-cells, which occupy the more median portions of the layers. About twice as many layers of secondary bast fibres can be counted as there are annual rings in the wood, which arises from the fact that, apart from the first two years, two such layers are pretty regularly formed in each year. The primary **medullary rays** in the wood are mostly two cells thick, but now and again even more; the secondary medullary rays are but one cell broad. They may be traced either right through the cambium and bast into the primary cortex, or only to a position in the bast equivalent to that in which the wood end of the ray is situated. The outer ends of the larger primary medullary rays broaden out funnel-wise, and separate two keel-shaped masses of bast. The central cylinder is surrounded by the living green primary **cortex**. In the outer parts of the medullary rays, and in the primary cortex, are numerous crystallogenous sacs. Still more externally is a layer of **collenchyma**, chlorophyll-containing, and

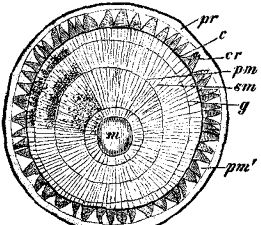


FIG. 58.—Cross-section through a stem of *Tilia parvifolia* in its fourth year's growth. *pr*, primary cortex; *c*, cambium ring; *cr*, bast; *pm*, primary medullary rays; *pm'*, outer broadened (funnel-shaped) end of a primary medullary ray; *sm*, secondary medullary ray; *g*, inner limits of the current year's ring (× 6).

readily recognisable by the glistening white walls, strongly thickened, especially in the angles. Finally the surface of the stem is occupied by a regularly-developed **periderm**, the flat cells of which, proportionally to their age (*i.e.*, from within outwards) are more and more brown in colour.—A general idea of the structural arrangement can be obtained from the accompanying somewhat diagramatic view of the cross-section of a four-year-old stem of the lime, the description of which is given at the foot of the figure (Fig. 58).

For a closer study of the wood and bast of *Tilia* it is again desirable to prepare *cross-sections* of alcohol material cut from the thickest available stems; and it is an advantage to lay the pieces selected for work for a few days in a mixture of equal parts *alcohol and glycerine. We will examine them in iodine-glycerine.* All the elements will be found to be considerably larger than in the thin twigs. The **wood** of the lime tree consists of vessels (ducts), tracheïdes, wood-fibres, and wood-parenchyma (Fig. 59). The ducts are striking from their considerable breadth (*m*). They, and also the tracheïdes (*t*), so far as they are in contact with other ducts and tracheïdes, show bilaterally-bordered pits. The wood-fibres (*l*) are noticeable for their very sparse and exceedingly slender pits. Amongst all these elements the wood-parenchyma cells (*p*) are readily distinguished by the protoplasm, and occasionally starch grains, which they contain. In the **bast** we at once notice the bast-fibres (Fig. 60, *l*);

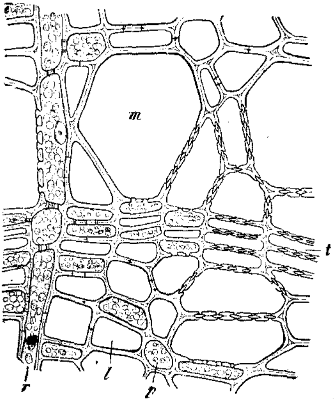


FIG. 59.—Cross-section through the wood of *Tilia parvifolia* (alcohol material). *m*, a wide-pitted duct; *t*, tracheïdes; *l*, wood-fibres; *p*, wood-parenchyma; *r*, medullary ray (× 540).

parenchymatous cells adjoining them (*p*) which mostly contain the abundant starch, but on the inner margin of the bast-fibres are *sometimes crystallogenous*; and the *wide-cavitied sieve-tubes* (*v*) with their companion-cells (*c*). These companion-cells are in most cases cut off singly and obliquely from a side of the sieve-tube, and hence are usually bounded by a bast-parenchyma cell or a medullary-ray cell. Where the section has chanced upon a sieve-plate (*v**), this is recognisable from its strong refraction, its sieve-pores, and its yellow-brown colour under the action of the iodine. In *Tilia* also we can determine that

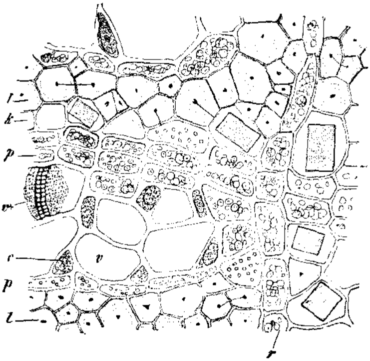


FIG. 60.—Cross-section through the bast of *Tilia parvifolia* (alcohol material). *v*, sieve-tubes, at *v** exposing sieve-plate; *c*, companion-cell; *p*, bast-parenchyma; *k*, crystallogenous cell; *l*, bast-fibre; *r*, medullary ray (× 540).

at a little distance from the cambium the sieve-tubes and companion-cells are empty. Traversing the bast the medullary-ray cells are at once recognisable from their arrangement and contents.

In the *radial longitudinal section*, which we also examine in iodine-glycerine, we first of all note the big **vessels** and **tracheïdes,** which besides the bordered pits upon their walls, also have thin spiral bands as an innermost thickening layer. The pitted ducts and the tracheïdes are connected by every intermediate form, and only the circumstance whether the end

wall is perforated by a round opening, or closed by bordered pits, determines whether the element in question is to be distinguished as a *vessel or a tracheïde*. The *greater proportion of the* wood is built up of long-pointed **wood-fibres**, the walls of which are provided with small cleft-like pits obliquely rising to the left, *i.e.*, following a left-handed spiral. Like vessels and tracheïdes, the wood-fibres also are without protoplasmic contents. While, however, vessels and tracheïdes serve for the conduction of water, the wood-fibres in fresh wood contain air, and only serve, therefore, to enhance the mechanical stability of the woody mass. They are to be reckoned rather as a portion of the stereome. The **wood-parenchyma** cells follow one another in uninterrupted *vertical rows; they are short, separated by simply pitted* walls, with protoplasmic contents and usually, also, filled with starch. The **medullary rays** (Fig. 61) cross the elements of the woody mass, as bands of very varied height. The edges of these medullary rays are *composed of* shorter or higher cells (*tm*), and we can see that it is these latter alone which are connected with the vessels by means of numerous large pits. Unilamellar medullary rays can be composed exclusively of the higher cells. The medullary-ray cells connected with the vessels by means of pits are marked by poverty in starch.

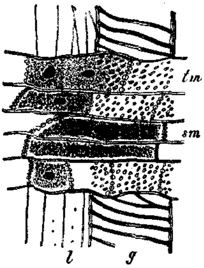


FIG. 61.—Fragment from a radial longitudinal section of the wood of *Tilia* with a small medullary ray. *g*, vessel; *l*, wood-fibre; *tm*, medullary-ray cells, connected by pits with the water-route; *sm*, conducting medullary-ray cells (× 240).

In the **bast** we note, above everything, the very long shining **bast-fibres**; we again find the starch-containing, and also the crystallogenous cells of the **bast-parenchyma**; *then the* **sieve-tubes**, the tangentially-inclined sieve-plates of which are cut up into separate sieve-areas. The narrow **companion-cells** are distinguished by their rich contents. Outside the bast, and if the stem be fairly young, we note the **collenchyma** with the white thickened angles, and the **cork-cells**, which have exactly the same form in longitudinal as in cross-section.

The *tangential longitudinal section* through the wood shows

us the same elements as in the radial section, but the medullary rays are cut in section, and from this point of view appear spindle-shaped (*r*). They are of very various heights, and may be unilamellar through their whole height, or multilamellar in the middle. The **intercellular spaces** are almost *completely* wanting at the sides of the unilamellar medullary rays; in the multilamellar they are only formed between the inner cells. The *tangential longitudinal section through the bast shows as far as the medullary rays are concerned the same relations, and can* also serve, just like the tangential section of the wood, to demonstrate to us the serpentine course which must be *taken by the elements* which are displaced by the medullary rays.

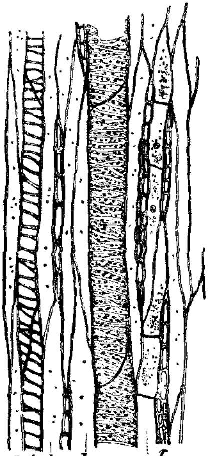


FIG. 62.—Tangential longitudinal section of the wood of *Tilia*. *m*, pitted duct; *t*, spirally-thickened tracheïde; *p*, wood-parenchyma; *l*, wood-fibre; *r*, medullary ray (× 160).

Chlorzinc iodine stains the lignified tissue yellowish-brown, the cambium violet. In the bast is shown a beautiful alternation of the violet thin-walled zones, and the bright yellow thick-walled bast-*fibres*. The *elongated medullary* rays of the primary cortex are violet, the cork is reddish-brown. Corallin stains the wood cherry-red, the bast-fibres quite a strikingly beautiful bright rose-red. The sieve-plates stand out clearly, even in the *cross-section*, by their *foxy*-red colour.

Isolation of Tissue-elements by Maceration.—It is always a matter of some difficulty for the beginner to make out with accuracy the individual components of complicated structures, such as are shown in longitudinal sections through the wood, particularly of secondary wood; and we will endeavour to supplement our knowledge by using another method, the so-called Schultze's *treatment* by "maceration". For this purpose we place in a wide test-tube a few crystals of chlorate of potash, and pour in enough nitric

acid to completely cover them,[1] then lay on this fluid *longitudinal sections*, not cut too thin, of the tissue to be investigated, and warm it over a flame until active evolution of gas ensues. The process should not be carried on in the same room with a microscope, as the vapour evolved would injure it. We allow the reagent to work for a few minutes, till the sections appear quite white, and then pour the whole into a large evaporating dish, or saucer, full of water. The floating sections should be removed from this, by means of a glass rod, into another vessel filled with water, so as to wash them; and from thence into a drop of water upon an object-slide. The preparation can here be torn to pieces with needles, and the individual elements separated. If the reagents have worked rightly, the pectose middle lamellæ between the elements will be dissolved, and their separation easy to complete. All the elements which previously had been studied in combination, will now be found isolated nnder the microscope. They are usually well preserved, except that the lignin, by which the cellulose in the wood had been chemically altered, having been more or less completely removed, they for the most part stain violet with chlorzinc iodine.

Instead of chlorate of potash and nitric acid, maceration can be carried on by means of chromic acid. A concentrated aqueous solution is used, and allowed to act for only a short time, perhaps half a minute. Then wash with a large quantity of water. The sections must not be too thick, or they cannot be readily disintegrated. In general this method is inferior to that of Schultze. With herbaceous plants, when the middle lamellæ between the elements are not lignified, hydrochloric acid and alcohol can be used for maceration; a mixture of one part hydrochloric acid and from five to three parts of alcohol. This fluid should act upon the sections for about twenty-four hours; then wash the sections, treat them with an alkali, such as 10 per cent. solution of ammonia, and the cells can be separated from one another by slight pressure.

In the case of *Tilia* we will preferably use Schultze's method,

[1] If the proportions of these reagents are not right, red fumes of nitrous acid gas will be given off, and the tissue may be entirely dissolved. W. R. McNab has suggested a fluid composed of two drachms nitric acid (of sp. gr. 1·10) and three grains of chlorate of potash, in which the tissue should be kept for a fortnight, when the constituent elements can be separated (*Trans. Edin. Bot. Soc.*, xi., 293).

tearing up the macerated sections longitudinally with needles under the low power of the microscope. The preparation will then show the especial preponderance of the wood-fibres (Fig. 63, *A*, *B*). The swelling of the walls causes the pits to appear still more minute; they show as oblique clefts. The short parenchymatous cells, re-

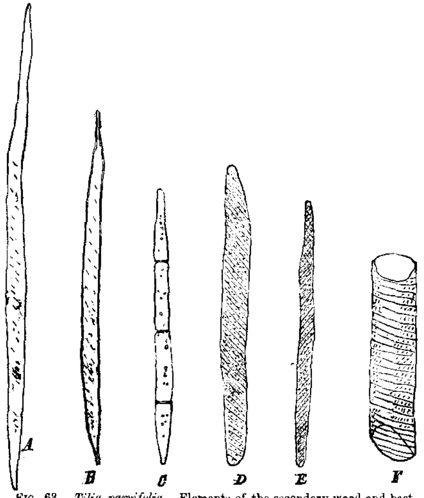


FIG. 63.—*Tilia parvifolia.* Elements of the secondary wood and bast, isolated by maceration. *A* and *B*, wood-fibres (libriform); *C*, wood-parenchyma; *D* and *E*, tracheïdes; *F*, segment of a duct; *G*, bast-fibre (× 180).

cognisable by their contents, lie scattered between the wood-fibres, and are either separated, or, corresponding with their origin from cambial cells, still joined into short threads (*c*), which resemble in outline the wood-fibres, and lie scattered between them. We find, further, but in smaller number, the spirally-thickened tracheïdes, in outer contour either more resembling the wood-fibres (*E*), o

more approaching the ducts (*D*); finally the ducts themselves, either separated into segments (*F*), or else forming long tubes. The very long and narrow-cavitied bast-fibres (*G*) are very noticeable in the preparation.

Length of the Tracheæ.—If for an example a twig of the Alder, *Alnus glutinosa*, eight or ten years old, be plunged under water, and, from its middle portion, a piece of about four inches in length be cut while still immersed, this piece can be used for the purpose of studying the length and continuity of the vessels. To the *upper* end of this piece of twig, *i.e.*, the end which was nearer the leaves, can be attached a glass-tube, about two and a half inches long, by means of a piece of india-rubber tubing, the other end of the glass-tube being connected with an air-pump or an aspirator. The lower end of the piece of twig is now immersed in a fluid composed of one part commercial "dialysed iron" (officinal liquor ferri oxychlorati) in three parts water. By the *gentle* action of the pump, or by the aspirator, this brown fluid can be sucked into the piece of twig, and it will be seen that the fluid which flows out into the glass-tube is colourless. The action may be continued gently for about an hour. Oxychloride of iron is a colloid body, and therefore diffusible neither through membranes nor through the closing membrane of bordered pits; hence it penetrates into each vessel only so far as the first closing, diaphragm-like wall; and so long as the brown fluid does not appear in the glass-tube it is clear that it has not passed through the length of the twig.

When injection with the iron is judged to be sufficiently complete, the lower end of the twig can be removed to a solution of ammonia (one part official spirits of sal ammoniac, three parts water), and the suction again resumed until the fluid which is drawn out at the upper end strongly smells of ammonia. All the iron salt will then have been deposited as a reddish-brown precipitate; and both with a lens and with microscopic sections, transverse and longitudinal, the distribution of this precipitate in the piece of twig can be studied.

Precisely similar experiments can be performed with *Tilia*.

Tyloses, or Tracheal Plugs.—In the vessels of especially the older wood of many stems and roots are to be found, sometimes occasionally, sometimes systematically, plugs of intruded cells, completely stopping the cavity, known as **Tyloses**, or, as we may call them, **Tracheal plugs.** We will study them in the wood of

the Bastard Acacia (*Robinia Pseud-Acacia*), in which they occur with remarkable regularity and frequency. Selecting a piece of old branch, say ten or twelve years old, we remove from it peripheral strips, penetrating into the wood to about the depth of the fourth year, and can discard the remaining central portion. These strips can be preserved in alcohol, but must then be treated, before working, with glycerine-alcohol to soften them. *Cross-sections* can be taken passing from the cambium inwards, and examined first with a low power. We shall soon note that the broader vessels, from about the third year's ring inwards, are filled with a thin-walled tissue (Fig. 64). We can generally find vessels in which the origin of this tissue can be followed. We can see bladder-like structures arise from one or several places on the wall of the trachea, and protrude into the cavity (at *a*). It is these bladders which, enlarging and pressing upon one another, and flattening on their contact sides, fill the whole vessel with tissue. At specially favourable places in the cross-section we can determine that it is the adjoining wood-parenchyma cells which protrude, bladder-wise, through individual bordered pits into the cavity of the vessel. It is the unlignified closing membrane of a unilaterally-bordered pit which is concerned in the process.—These plugs are found in the stems of a few Monocotyledons, many Dicotyledons, and occasionally in Conifers. In woody roots they are rare, but common in herbaceous roots, as, *e.g.*, *Cucurbita*.

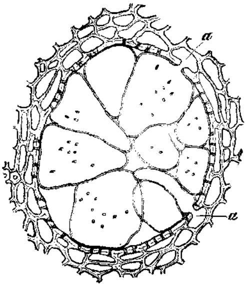


FIG. 64.—A vessel filled with a Tracheal plug, together with the adjoining cells, from the duramen of *Robinia Pseud-Acacia*, in cross-section. At *a* and *a*, the connection of the plug cells with their mother cells can be seen (× 300).

CHAPTER XII.

THE CENTRAL CYLINDER (STELE) AND SECONDARY THICKENING OF ROOTS.

PRINCIPAL MATERIALS USED.

Roots of Onion (*Allium Cepa*), or of Hyacinth, or of *Acorus Calamus*; fresh or in alcohol.

Roots of Flag Iris; alcohol material.

Roots of *Ranunculus repens*; in alcohol.

Roots of *Taxus baccata*, *e.g.*, from seedlings; alcohol material, softened with alcohol-glycerine.

PRINCIPAL REAGENTS USED.

Concentrated sulphuric acid—Potash—Aniline blue.

Root of Allium Cepa.—The structure of the **central cylinder** of roots we will first study in the root of *Allium Cepa*, the garden Onion. Abundant material for investigation can be at any time secured by allowing an onion to grow in water in a hyacinth glass. Figure 65 shows us a *cross-section* from the base of a strong **adventitious root** thus obtained. The epidermis, or **piliferous layer** (so called from bearing the root-hairs), and the very thick primary cortex, are left out of the drawing, but of the latter we see the cells (*c*) which bound the **bundle-sheath**, or **endodermis** (*e*). This endodermis shows a characteristic dark shading upon the radial walls, which is produced by the wavy flexure of a median strip of the walls, which is also lignified. Such an endodermis is always unilamellar; it arises from the innermost layer of the cortex. In old roots the endodermic cells are thickened on their inner and radial walls in U-form, and these thickening layers disguise the original structure of the radial walls. The centre of the vascular cylinder is occupied by broad **scalariform vessels** (*sc*). If the root is not old enough the

central, and perhaps also the adjoining, vessels are thin-walled, and not fully developed. Adjoining the one or more central vessels are almost always six narrower scalariform vessels (*sc**); to each of the last succeed a group of quite narrow **spiral** and **annular vessels**, or vascular tracheïdes, which constitute the **protoxylem** (*sp*, *sp + a*). The size of these elements, therefore, diminishes from within outwards, and it is the spiral and annular protoxylem which lies outermost; an exactly opposite condition to that in the stem, and which has arisen from the twisting of the

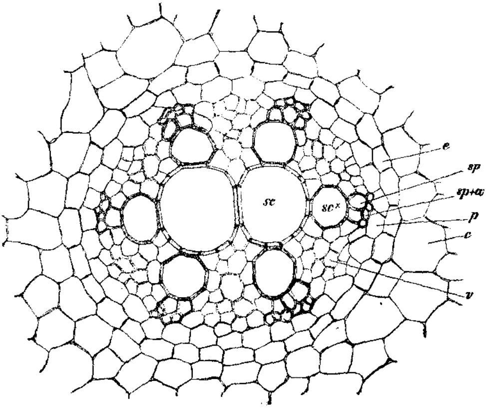


FIG. 65.—Cross-section of the base of a strong adventitious root of *Allium Cepa*. *c*, cortex; *e*, endodermis; *p*, pericycle; *a*, annular vessels; *sp*, *sp**, spiral vessels; *sc* and *sc**, scalariform vessels; *v*, bast (× 240).

xylem bundle through 180 degrees. The xylem strings are in this case united into a six-rayed star, and the central cylinder is therefore described as "*hexarch*". The **bast** strings (*v*) alternate with the xylem, a relation which is universal for the central cylinders of roots. Wood and bast are separated from one another by a layer of parenchymatous ground-tissue cells—the **conjunctive tissue.** The bast portions can be recognised by the white shining walls of their cells; they consist of some sieve-tubes and companion-cells, which latter are, however, not to be dis-

tinguished with certainty in cross-section from the sieve-tubes. From the endodermis the wood and bast are separated by a single layer of cells, the **pericycle** (*p*). This pericycle, therefore, occupies the same relative position in roots as in stems, and constitutes the outermost layer of the central cylinder or **stele.** —In concentrated sulphuric acid the entire cross-section is dissolved, with the exception of the epidermis, the layer of cells underlying it, the endodermis and the vessels. These last have stained a beautiful yellow. The endodermis, which from the action of the sulphuric acid will have partially turned over, shows the middle band in the radial walls beautifully undulated. In the outermost cortical layer also, adjoining the epidermis, a similar appearance is, however, to be observed; and if we examine again our earlier preparation, we shall assure ourselves that in this case also the radial walls show a dark shading. The cells in question are also firmly united together, and form, therefore, a kind of outer endodermis, which has been termed **suberose layer** or **exodermis.**

The *longitudinal section* shows the vessels with the thickenings already referred to; and with aniline blue the sieve-plates of the sieve-tubes can easily be made visible through staining sky-blue. From the sieve-tubes their companion-cells can now be distinguished by their abundant contents and their smaller length. The wavy middle band of the radial walls of the endodermis, seen now in surface view, shows as a ladder-like thickening.

Root of Hyacinth.—The roots of the Hyacinth (*Hyacinthus orientalis*), similarly grown in water, may be used as an alternative to those of the Onion, with which they agree in all essential points. The number of vascular bundles usually varies between six and eight.

Acorus Calamus.—The root of the Sweet Flag, (not uncommon by the sides of streams and ditches in the eastern and midland counties, and the rhizome of which is used in perfumery), will serve to further inform us as to root-structure. The *cross-section* of a piece of a fully-developed root (Fig. 66) shows that here the xylem rays (*s*) of the central cylinder, do not coalesce in the centre of the cylinder, but are usually, to the number of eight, arranged in an interrupted ring, while the middle is occupied by a **pith.** The large vessels lie, as in *Allium*, towards the interior, the small ones towards the periphery. The bast strings (*v*) alternate, as usual, with the wood rays. They are separated

laterally by a single or double layer of parenchymatous ground-tissue cells (conjunctive), and, outwards, are separated from the endodermis (*e*) by a unilamellar pericycle (*p*). The endodermis consists of flattened, thin-walled cells. The endodermis, the pericycle, and all the other ground-tissue cells in the central cylinder, are usually thickly filled with starch; whence the bast strings, which contain no starch, show up specially clearly in the figure. The cells of the inner cortex are separated into unilamellar layers by numerous air-canals. In the periphery the cortical cells are crowded together into a firm, strong, multilamellar sheath. The outermost, hypodermal, cortical layer consists of radially elongated cells, and here, as in other roots, forms an exodermis, which persists, whilst the epidermis itself dies and is destroyed. If potash solution is run in, the starch disappears out of the cells, and the existence of dark shadings on the radial walls of the endodermis can be easily proved. On this endodermis, as treatment with sulphuric acid shows, only the band which forms the shadings is cuticularised, while of the exodermis the whole cell-wall is. In an earlier stage, however, the exodermis, like the endodermis, is suberised only in median strips of the radial walls. The complete suberisation is connected with the protective function of the layer; the epidermis, cut off by it from the living part of the root, becomes exfoliated. The cells of the exodermis contain resin.—By virtue of the suberised band on the radial walls of the endodermis, the central cylinder is rendered air-proof against the intercellular passages of the cortex; but in order, however, that the passage of fluids between the central cylinder and the cortex may still remain possible, the tangential walls are not suberised.

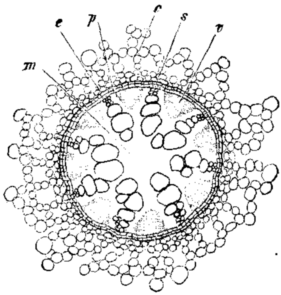


FIG. 66.—Cross-section through the root of *Acorus Calamus*. *m*, pith; *s*, wood; *v*, bast; *p*, pericycle; *e*, endodermis; *c*, cortex (× 90).

Root of Iris.—A *cross-section* through the root of *Iris florentina*

or other Flag Iris, shows in its axial vascular cylinder the closest resemblance to *Acorus*, but the endodermis is somewhat differently constructed (Fig. 67). The cells of this (*e*) are unilaterally thickened on their side towards the interior, into the form of the letter U, and the thickening is beautifully stratified. At isolated points a single unthickened cell occurs, and it can be determined that, wherever present, such a cell (*f*) always lies in front of a ray of the wood. These cells we will call "**passage-cells**"; they are permeable, and maintain the connection with the surrounding cortex (*c*). In concentrated sulphuric acid the thickening layers of the endodermis swell and are dissolved; only the lignified **middle lamellæ**, forming a delicate layer around the endodermic cells and also around the passage-cells, remain. Similarly, the middle lamellæ between the vessels and in the pith are not dissolved, and form a delicate brownish-yellow network.—A *tangential longitudinal section*, which skirts the endodermis, shows us that the longitudinal strip of this endodermis which lies outside the xylem strands consists of an alternation of long thickened cells and of short unthickened passage-cells, the latter with abundant cell-contents. Here and there two such passage-cells follow one another.

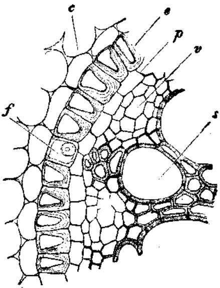


FIG. 67.—Part of a cross-section through the root of *Iris florentina*. *e*, endodermis; *p*, pericycle; *f*, passage-cells; *v*, bast; *s*, vessel in the wood; *c*, cortex (× 240).

Where the median strip of the radial walls of the endodermis is cutinised, the layer would probably entirely exclude the air which is present in the cortical intercellular spaces from penetrating to the central cylinder, where, by obtaining access to the cavities of the vessels, it might act as an obstacle to the passage of the water; while, on the other hand, water itself can freely pass through the non-cuticularised inner and outer walls. This would be of especial importance for endosmotic purposes in the root-hair, or absorbing region, of the root. In older parts of the root, where passage of water through the endodermis is no longer of importance, the cells may be cuticularised, or thickened,

throughout, without disadvantage. If the thickening takes place early, then water transit may be effected by means of the passage-cells, which, as we saw above in Iris, are placed opposite to the groups of water-conducting vessels in the central cylinder.

Root of Ranunculus repens.—The roots of Dicotyledons are less favourable for study than those of Monocotyledons; but now that we have obtained an insight into these latter, it will not be difficult correctly to interpret the former. We prepare a *cross-section* from the base of a strong adventitious root of the runner of the Creeping Buttercup (*Ranunculus repens*) abundant everywhere by roadsides and in pastures, and as a garden weed in damp soils. The central cylinder, or stele, does not appear so sharply defined towards the cortical tissue as it is in Monocotyledons, but with careful observation we shall find, at the junction of the two, the endodermis, marked with its dark shading; for a thickening endodermis, so common in Monocotyledons, is more exceptional in Dicotyledons, and this absence stands manifestly in relation with the power of secondary growth which their central cylinder in general, though not in this particular case, possesses. According to the strength of the root, the wood in the central cylinder is in four or five rays; the great vessels here also lie towards the centre, the small ones outside them. In Monocotyledons an innermost vessel is often distinguished by its exceptional size; in Dicotyledons this is seldom the case, and is not to be observed in *Ranunculus*.[1] The xylem rays in *Ranunculus* extend to the centre of the stele, and there coalesce more or less completely with one another. Yet, if at all, the innermost vessels are only fully formed quite late, and remain mostly in the state of thin-walled, elongated cells. The bast bundles alternate with those of the wood in the ordinary way.

Roots of Vascular Cryptogams.—The roots of Vascular Cryptogams are constructed upon the same fundamental type as those of Phanerogams.

Secondary Thickening in Taxus baccata.—We will follow out in *Taxus baccata* (the Yew) the processes which lead to the secondary increase in thickness of those roots of Dicotyledons and Gymnosperms which are capable of it. For this purpose we procure a piece of root with young uninjured branchlets. We take

[1] In the adventitious roots upon the rhizome of *R. repens*, according to De Bary, *Comparative Anat.* (Eng. trans., pp. 355-6, and Fig. 165), the axis of the cylinder is occupied by one large vessel or pitted duct.—[Ed.]

a *cross-section* through a lateral root which is about 1 mm. thick, and at perhaps $\frac{2}{5}$ inch from the tip.[1] The surface is composed of a parenchymatous cortex at least ten cells thick. The outermost cell-layer of the cortex is not sharply defined, as a differentiated epidermis is wanting. The centre of the section is occupied by the central cylinder. This is surrounded by the endodermis, which consists of flat, thin-walled, corky cells, whose walls are brown, and whose diameter is manifestly inferior to that of the cortical cells. These cells show, on the radial walls, the characteristic dark shadings. Around the endodermis is developed a "**strengthening layer**," likewise unilamellar; its cells have the width of the other cortical cells, but have, however, their radial walls distinguished by a thick, shining, yellow ring. These annular thickenings coincide in adjoining cells, and therefore give in cross-section the figure of a very bi-convex lens. The central cylinder shows a diarch xylem in the form of a transverse plate (Fig. 68 *t'*); composed at its two ends of the narrow spiral vessels, and, for the rest, a band of broader tracheïdes, with bordered pits, of the kind characteristic of Coniferæ. On each side of the tracheïdes lies a layer, in the main double, of ground-tissue (conjunctive) cells, with narrow cavities, thin walls, and containing starch. To these adjoin the still smaller-celled tissue of the thin-walled bast, composed of sieve-tubes and protoplasmic bast-parenchyma. Lastly outside this bast are the starch-containing cells.

Now examine a *cross-section* about 1·3 mm. thick, and at about 1 inch from the root-tip, and we shall see that, on both sides of the plate of tracheïdes, the layer of the conjunctive ground-tissue, bounding the bast internally, has commenced to divide. It is converted into a layer of **cambium**, which henceforth produces internally tracheïdes, externally bast, and on both sides medullary rays. We shall see the results of the continued activity of this cambium layer in a root 2 mm. thick; the present structure is illustrated in the subjoined Figure 68. The cross-section shows the general relations already known to us; the cortex (*c*), the outer strengthening layer (*m*), the endodermis (*e*), and the central cylinder. The outermost cell-layer of the pericycle has in the meantime begun to divide by tangential walls, and forms a thin layer of **periderm** adjoining the endodermis. On both sides

[1] Seedling plants serve very well.—[Ed.]

of the median plate of tracheïdes (t') we see the inner inactive layer of the conjunctive ground-tissue (f), which may be considered equivalent to a pith; farther on the newly-formed and radially-arranged *tracheïdes* (t''), *with numerous interpolated medullary* rays.

It is easier to obtain information as to these relations if a little potash solution is added to the preparation. The spiral vessels (s) at the ends of the median plate stand out clearly, with dark outline; the tracheïdes with bordered pits (t') of this median

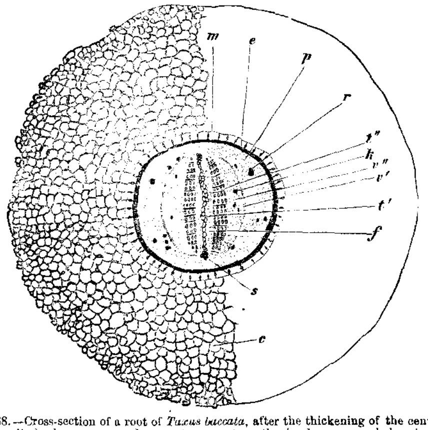


FIG. 68.—Cross-section of a root of *Taxus baccata*, after the thickening of the central cylinder has commenced. *c*, cortex; *m*, strengthening layer; *e*, endodermis; *p*, pericycle; *s*, spiral vessels; *t'*, primary band of tracheïdes; *f*, bands of ground-tissue; *t''*, *secondary tracheïdes with medullary rays*; *v''*, *secondary bast*; *v'*, crushed primary bast; *k*, cells in the secondary bast with crystals in their walls; *r*, resin-containing cells in the pericycle (× 42).

plate, as well as those of the secondary (cambial) growth (t''), are stained a beautiful yellow; the inner connective tissue (pith) remains colourless. The secondary xylem bands have a plano-convex contour; at their ends they taper off, but do not come near the spiral vessels. On the outer margin of the secondary xylem we find the cambium, and outside that the secondary bast (v''), which after treatment with potash appears colourless, but in

which, however, single cells (*k*) appear black. These are the cells in the walls of which crystals of oxalate of lime have become embedded. The primary bast (*v'*) is found, crushed, on the outer side of that which is secondarily produced. In the pericycle, after potash, far more clearly than before, single irregularly-placed cells are marked by their yellow-brown contents; they contain resin. The cork-layer, developed from the outermost layer of the pericycle, is coloured yellowish-green by the potash, the thickening rings of the strengthening layer a bright yellow. The endodermis has been crushed flat by the cork-layer.

If we now examine the *cross-section* through a still older root, about $\frac{1}{12}$ inch thick, which has already cast off its cortex, and shows a dark-brown surface, it will show us a completely-closed xylem ring; and the appearance would hardly be distinguishable from that of a cross-section of a stem of similar thickness, were it not that the position of the pith is occupied by the primary plate of tracheïdes. The surface is covered with the pericyclic cork-layer.

Longitudinal sections through the roots are necessary in order to determine that the median plate of tracheïdes consists of exactly the same elements as the secondary wood. We again find the spiral vessels at the edges of this plate, and determine that the cells of the endodermis have only small height, while those of the strengthening layer are far larger, and even surpass in height the contiguous cells of the cortex. With aniline blue we can identify the sieve-plates in the primary and secondary bast.

Dicotyledonous roots, as a rule, have stellately-arranged polyarch wood portions, instead of the two only (diarch) which are present in the Yew. In all those which thicken the same essential plan is, however, carried out; streaks of cambium appear on the inner side of each portion of bast; gradually the cambium streaks extend, and join into a ring, which encloses all the primary wood and excludes all the primary bast; and forms wood internally, bast externally, and medullary rays on both sides. In some cases the medullary rays formed opposite to the primary bundles of wood remain far broader than the others, and the ligneous mass continues to be irregularly stellate, the broadening rays of this mass, however, being alternate to the original rays of the wood; but as a rule these medullary rays are not distingnishable from the others, and the wood is formed in rings, with cambium, and outside that bast, just as it is in the diarch central cylinder of the root of the Yew.

CHAPTER XIII.

THE STELE OF THE PTERIDOPHYTA.

PRINCIPAL MATERIALS USED.

Rhizomes or petioles of *Pteris aquilina;* in alcohol.
Stems of *Lycopodium* sp., *e.g.*, *L. complanatum;* in alcohol.
Barren stems of *Equisetum arvense*, or other sp.; fresh, or in alcohol.

PRINCIPAL REAGENTS USED.

Aniline blue—Safranin.

The Rhizome of Pteris—Stelar Theory.—In our studies of the structure of stem and root of Phanerogams, we have used the word **stele** as being to all intents and purposes equivalent to the term **central cylinder**, in which sense Van Tieghem, who introduced the term, used it. The stele then consists, in the cases we have studied, of more or less numerous vascular bundles, variously arranged with regard to one another, but alike in that the whole system of bundles is surrounded by a **pericycle** (in which subsequent developments of the most diverse kinds may originate); and that the pericycle, the most external tissue of the stele, is very often cut off from the general body of the surrounding **cortex** by a specialised layer of this latter, the bundle sheath, or **endodermis**; though we have also seen that the endodermis varies very greatly in respect to its recognisability—sometimes very distinguishable (*e.g.*, most roots), sometimes forming a "starch layer," sometimes not recognisable as a separate layer at all. In most of the Vascular Cryptogams the stele presents itself to us under an aspect which differs greatly from that of Phanerogams, at first sight often simulating a vascular bundle; and we will take as our first object of study of the stele in their stems an exceedingly common fern, the Bracken (*Pteris aquilina*).

A *cross-section* through a stout creeping rhizome of the Bracken, examined with the naked eye or with a lens, shows it more or less elliptic in section, and covered externally by a thick rind of a dark brownish-yellow colour, which microscopic study would show to be an epidermis and a pseudo-sclerenchymatous layer, the aggregate colour in which is due to the natural bright yellow coloration of the cell-walls. Within this rind is the general mass of the ground-tissue, an opaque white in colour, and very sappy in fresh material. Two dark brownish-yellow bands can be seen in this, of unequal size, one nearly flat and the other arched, and dividing the general ground-tissue into an inner and outer portion, communicating between the ends of the bands; these are bands of **internal sclerenchyma,** and in some cases, especially near the point of branching of the rhizome, these bands join into one, of horse-shoe form. Both within and without these internal sclerenchyma bands, and embedded in the ground-tissue, will be seen what subsequent study will show to be the **vascular strings,** golden-yellow in colour, and each enclosed in a delicate brownish line. These vascular strings were formerly known as vascular bundles; they are **steles,** and, as we shall see, each is enclosed by a starch sheath, and an outer layer of cells of the ground-tissue constituting an endodermis. The steles, however, from time to time coalesce one to one, so that, if their longitudinal course were traced, they would be seen to form a very irregular network; hence while the rhizome is considered to be **polystelic,** the steles may be considered as **schizosteles** when they separate, or **gamosteles** when they coalesce. The stem of a very young fern only shows one central stele, and in this respect strikingly resembles the root of the same plant. If then each of these vascular strings is a stele, the general ground-tissue in which they are embedded is manifestly equivalent to that which we have called "cortex" in phanerogamic stems and roots. The term "cortex" is, indeed, sometimes applied to it in the Fern; but inasmuch as this term has acquired a specific meaning, as an external enveloping layer, or rind, the application of the same term to this case appears undesirable. I [ED.] have already said that in many Dicotyledonous stems the limits of the central cylinder are not recognisable; it does not follow that the limits are not present, but if we suppose for the moment that each vascular bundle of such a stem were in itself a stele, the cortex, medullary rays and pith would form a collective ground-tissue of a kind

strictly comparable to that which we see in the rhizome of the Fern.[1]

Stele of Pteris.—In *Pteris aquilina* the general relations of the steles are the easiest to make out, although the abundant sclerenchyma makes the material somewhat difficult to cut. Alcohol material is undoubtedly preferable, and *cross-sections* may

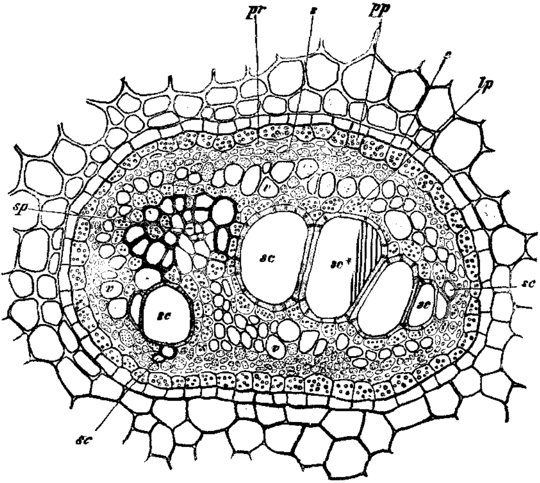


FIG. 69.—Cross-section through a stele from the leaf-stalk of *Pteris aquilina*. *sc*, scalariform vessels ; *sp*, protoxylem ; in the scalariform vessel *sc**, a piece of a ladder-like wall is broken through ; *lp*, wood-parenchyma ; *v*, sieve-tubes ; *s*, phloëm cells ; *pr*, protophloëm ; *pp*, starch sheath ; *e*, endodermis (× 240).

be cut from the rhizome not too far behind its growing end, or from the leaf-stalk of a leaf that is still young. In such sections the steles will be found already fully developed, while the characteristic (sclerenchymatous) thickening of the ground-tissue is still wanting. The structure of the stele is the same in rhizome as in leaf-stalk, and the accompanying Fig. 69, which shows us the
s cross-section of a stele from the base of a leaf-stalk, will serve to

[1] See note on page 181.

illustrate it. For considerations of space a small stele has had to be selected for representation; still, all the elements entering into its composition can be sufficiently well represented in the figure. The first things to attract attention are the great **scalariform vessels** (*sc*) *marked with elongated-bordered pits. For the most* part these are in contact with one another, and are surrounded as a whole by starch-containing **wood-parenchyma** (*lp*). At one spot on the small stele figured, or at two or many spots in larger steles, lie a few scalariformly or spirally-thickened, and partially-disorganised **protoxylem** elements. Vessels and wood-parenchyma together form the xylem of the stele. Well nigh surrounding this we see the wide openings of the **sieve-tubes** (*v*), and to these follow externally the cells of the **bast-parenchyma**, rich in protein contents. The periphery of the bast shows an interrupted layer of still narrower swollen **protophloëm** elements (*pr*). All these tissues are surrounded by a simple layer of starch-containing cells (*pp*), the **starch sheath,** which is surrounded by the thin-walled but starch-free and corky **endodermis** (*e*), which, in the comparatively young state we are studying, shows the dark shadings on the radial walls. The cells of the starch sheath and of the endodermis coincide with one another, and have a common origin. They both arise through the tangential division of the innermost layer of the general ground-tissue, the so-called **phloeoterma.** The phloeotermatic starch sheath here replaces the pericycle. At the two edges of the xylem strand the sieve-tubes are often wanting, so that wood-parenchyma and bast-parenchyma (or possibly even the protophloëm or the starch sheath) are in direct contact. Not infrequently the section will have skirted the scalariformly-perforated partition wall of a vessel, which may then have the appearance shown on the figure at (*sc**). The walls of the endodermic cells are very commonly torn in cutting, so that the stele is separated from the surrounded ground-tissue. The cells of the ground-tissue bordering on the endodermis are here and there strongly thickened, and then coloured yellow-brown.

The *cross-section* through the rhizome shows, under the deep-brown epidermis, a brown and cutinised parenchymatous tissue (falsely-called "cortical sclerenchyma") which further towards the interior is colourless and full of starch. This starch-containing ground-tissue is traversed by the steles, and by reddish-brown sclerenchyma fibres. These latter form bands between, and run-

ning more or less parallel to, the steles, which thus form an inner and an outer series. The outer steles are supported on their *outer side, immediately adjoining the endodermis, by similar* sclerenchyma fibres, which evidently have a mechanical function. In the interior of the leaf-stalk the relations are similar, excepting that here there is also a hypodermal ring of reddish-brown sclerenchyma fibres, which underlies the epidermis, and forms a true **cortical sclerenchyma.**

The *longitudinal section* through the rhizome, or the leaf-stalk, shows most prominently the scalariform vessels. The end walls of these are sharply inclined, with ladder-like bordered pits, partially broken through. It is now easy to determine that on the side walls which separate two vessels the horizontally elongated pits are bordered on both sides, and that the **closing membrane** possesses a thickened **torus,** but on the wall of those vessels which adjoin a wood-parenchyma cell the border is only unilaterally developed (on the side towards the vessel), and that the closing membrane has no torus. The longitudinal section may also have cut through one or other of the spiral protoxylem elements, and the sieve-plates of the sieve-tubes may also, with very careful examination, be disclosed, being made more recognisable by numerous refractive granules. We can make the sieve-plates somewhat clearer with the aid of aniline blue, and determine that the terminal sieve-plates are very oblique, and divided by thickened bands into numerous sieve-areas. Besides these, the lateral walls of the tube also bear roundish sieve-pits. Near the sieve-tubes can be recognised the bast-parenchyma with abundant protoplasmic contents and nucleus; in contact with the vessels, the starch-containing, comparatively short cells of the wood-parenchyma; while of much the same shape as these are the starch-containing cells of the peripheral starch sheath. The red-brown, long, pointed sclerenchyma fibres of the ground-tissue show delicate pits in their walls.

Stem of Lycopodium.—A somewhat higher degree of complexity is shown by the axial stele of the species of *Lycopodium* (Club-mosses); but the structure will not appear so difficult after we have made a careful study of the stele of the Fern. In *Lycopodium* we have, in fact, a coalescence of a number of steles, each like that of the Fern, into a **gamostele.** For study we select *Lycopodium complanatum*; but any other species will serve equally well, since in all species of *Lycopodium* the relations

in question recur with unimportant deviations. Our task will be somewhat lightened by staining the *cross-section* with aqueous solution of safranin, and the accompanying sketch (Fig. 70) will give us some general information. In the cross-section of *Lycopodium complanatum* we find most externally the **epidermis** (*ep*); then the **cortex**, the cells of which at first have wide cavities, but farther towards the interior diminish in width and increase in thickness, and so form a firm **sclerenchymatous sheath** (*ve*). The outer cells of this sheath have stained more cherry-red with the safranin; the inner, strongly thickened cells more rose-red. The thickened elements of the cortex end suddenly, and there succeed two or three layers of polygonal cells, somewhat tangentially elongated, united uninterruptedly, and staining cherry-red (*vi*). These cells are cuticularised, and resist sulphuric acid well. Farther within follow several layers of similarly wide-cavitied isodiametric cells, often containing starch, with glistening, as it were swollen, walls (*pp*). With relatively short action these have not been stained, but with longer are orange-red. They form the innermost layer of the cortex. We now notice the xylem strands, beautifully stained cherry-red. They consist of broad **scalariform tracheides** (*sc*), in lateral contact with one another, and, at the thin edges, of narrow **protoxylem** elements. True vessels with perforated end walls are not present in *Lycopodium*, though, for the purpose of comparison with other vascular plants, we may consider these xylem strands to be vascular. The flat xylem bands in *Lycopodium complanatum* run within the central cylinder, and more or less

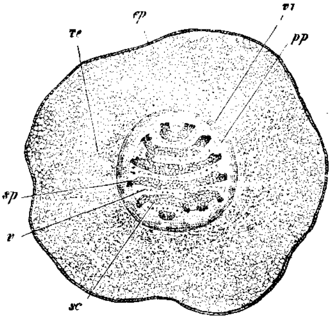


FIG. 70.—Cross-section through the stem of *Lycopodium complanatum*. *ep*, epidermis; *ve*, outer cortical zone; *vi*, inner cortical zone; *pp*, innermost cortical zone; *sc*, scalariform tracheïdes; *sp*, annular and spiral protoxylem; *v*, bast (× 26).

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parallel to one another. On the one side they are somewhat concave, on the other proportionately convex; and we can determine, if we take note of the natural position of the creeping stem upon the earth, that the bands appear parallel to the surface of the earth, and with the concave side always turned upwards. The small steles of the leaves, after they have entered into the central cylinder, join on to the edges of a xylem band, just as in the ferns. The xylem bands not infrequently anastomose, an example of which can be seen in the lower bands of the sketch (Fig. 70). In the erect stems of *Lycopodium Selago* the whole of the xylem bands are, in fact, combined into the form of a star. The xylem bands are surrounded by a single layer of thin-walled, narrow-cavitied cells, which, as in the ferns, we can designate wood-parenchyma cells. Midway between the bands formed by the wood lie cells with white, strongly refractive walls; they have narrow cavities, but a median row is distinguished by somewhat broader cavities. These form the **bast**; the larger elements in this latter are the sieve-tubes (*v*). In specially favourable cases of safranin staining, the walls of the sieve-tubes are rose-red, while the other elements of the bast remain colourless. At the edges of these bands of sieve-tubes the **protophloëm** elements are distinguished by the narrowness of their cavities. At the inner limits of the cortex the central cylinder (gamostele) can be easily broken away in section-cutting.

Longitudinal sections show us: most externally, the epidermis; then, the broad cortical cells running obliquely towards it; further, the sclerenchyma fibres of the outer cortical sheath; after this the inner cortical sheath of elongated parenchyma; the innermost cortical sheath with white, thicker walls, and end walls situated obliquely; the scalariform tracheïdes, and the narrow, in part very greatly stretched, annular and spiral elements of the protoxylem; finally, also, the elements of the bast. These last consist of very long cells, the end walls of which are more or less oblique. With the aid of aniline blue it is possible, though very difficult, to recognise the comparatively small, oblique sieve-plates. Only the broadest elements in the bast are sieve-tubes, the much more numerous narrow elements, filled with refractive granular contents, form the protein-containing bast-parenchyma.

Stem of Equisetum.—There are, however, also, Pteridophytes whose stem structure shows collaterally-constructed vascular

bundles, arranged annularly in a central cylinder, as in Gymnosperms and Dicotyledons. This is the case with *Equisetum. E. arvense* (the common Horsetail), can be obtained pretty well everywhere, and will be used therefore for a brief study of its essential structure. A *cross-section* shows a hollowed stem, the hollow (*m*) formed lysigenously, by the breaking down of tissues, and not infrequently containing water; a ring of vascular bundles of very reduced dimensions, each with an air-passage (*cl*); surrounding the ring is an endodermis (*e*); a thick cortex with larger air-passages (*vl*) alternating with the smaller ones of the vascular bundles; the exterior of the stem showing alternating ridge and furrow, the ridges with strings of sclerenchyma fibres, (*hp*) between which the chlorophyll-containing cortical tissue (*ch*) extends to the epidermis, and here stomata are found (*st*); while the median portion of each furrow also shows a sclerenchyma string (*hp*). The air-passages in the cortex underlie the furrows, and hence are called vallecular passages; those in the vascular buudle underlie the ridges, and hence are called carinal.

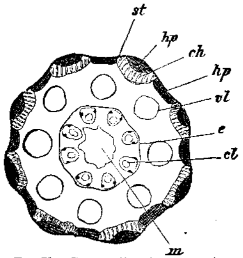


FIG. 71.—Cross-section through an internode of a barren shoot of *Equisetum arvense* (× 11).

Directing our attention more closely to the vascular bundles, we see that they are collaterally constructed; wood towards interior, bast towards exterior (Fig. 72). In the wood the water-containing carinal intercellular passage is very recognisable (*cl*); it is surrounded by thin-walled xylem parenchyma. Into the intercellular passage isolated rings project, derived from the stretched and torn annular tracheïdes of the protoxylem; other much stretched vascular tracheïdes are close by them. The xylem, apart from these vestigial remains, is situated at the sides of the bast, and consists of a number of unstretched resistant vascular tracheïdes, thickened annularly and spirally. The bast shows no recognisable alternation of broad and narrow elements, but none the less consists here also of sieve-tubes and bast-parenchyma, these latter recognisable by their abundant contents, and perhaps recognisable nucleus. Externally are the narrow protophloëm

elements. The vascular bundle is bounded externally by a starch-containing layer of cells (*am*) which is to be recognised as a **pericycle.** Within and laterally the bundle is clad with ground-tissue cells, devoid of intercellular spaces, though not otherwise specially marked. To the pericycle succeeds the ***endodermis*** (*e*) which therefore, like the pericycle, surrounds the entire ring of bundles. The endodermis is unilamellar, and as a rule shows clearly the dark shadings on the radial walls; sulphuric acid shows these up sharply as cutinised bands, while the rest of the tissue generally (except the rings, etc., of the xylem) are left colourless and gradually dissolved. Outside the endodermis is a large-celled cortex, having large **vallecular air-passages,** on radii alternating with the bundles. The surface of the stem shows projecting ridges; these ridges lie opposite the bundles, the epidermis being here devoid of stomata and protected by strings of sclerenchyma fibres. Under these strings lies the chlorophyll-containing tissue, loose parenchyma, which extends on both sides of the string to the epidermis. Here, on the slopes of the ridges, are found the stomata, while the middle portion of the furrow has again an epidermis devoid of stomata, and with a sclerenchymatous hypoderma, though more feebly developed.

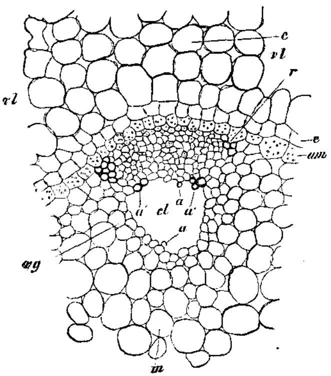


FIG. 72.—Part of a cross-section through the internode of a barren shoot of *E. arvense,* showing a vascular bundle and the surrounding tissue. *a*, isolated rings from vascular *tracheïdes*; *a′*, *crushed annular tracheïdes, representing the* protoxylem; *r*, annular tracheides at the sides of the bast; *cl*, carinal air-passage; *vg*, vascular bundle sheath; *m*, pith; *am*, starch sheath (pericycle); *e*, endodermis; *c*, cortex; *vl*, portion of a vallecular passage (× 90)

The physiological relations of these tissues are easy to deter-

mine. The stability of the slender stem is secured by the stereome, in the form of the chief sclerenchyma strings, being removed as far as possible outwards into the ridges of the stem, while they are aided by secondary ones which occupy the furrows, and not merely stiffen the epidermis here against "buckling," but protect the underlying vallecular passages. The principal sclerenchyma strings cover the assimilating tissue, which reaches the surface at the (mechanically) most protected places, *viz.*, on the flanks of the ridges. We have here, therefore, an admirable compromise between the antagonistic needs of the assimilating and mechanical system; the former of which should be near the surface, so as to expose its elements to light and air, while the latter to secure against flexure should be placed as peripherally as possible. The endodermis for its part makes the central cylinder air-tight against the air-containing tissue of the cortex; the cuticularised bands on the radial walls particularly aiding this, and preventing the formation of intercellular spaces.

Examination of the exterior of the stem shows that on successive internodes the ridges alternate with one another; hence the sclerenchyma strings also change their position at the nodes, being connected together by branches. Considered, therefore, as a whole this external mechanical system of the stem constitutes a lattice-work of vertical girders with very short tie-rods, and is primarily intended to resist flexure.

The structure of the roots of Pteridophytes is, in essentials, like that of Phanerogams. The periphery of the central cylinder of the root agrees, however, with that of the fern stele which we have studied, in that it is covered by a double layer of Phlœoterma, of which the inner layer constitutes the pericycle, and the outer, the endodermis.

NOTE ON THE STELAR THEORY.

So far as concerns the stems of the Vascular Cryptogams, Van Tieghem's "Stelar theory" is now in the "melting pot". As a morphological generalisation, however, it has great value; and I see no present reason for modifying the text of this chapter. What are called "steles" in the account of *Pteris* are by many again described under their pre-stelar name of "concentric vascular bundles".—[Ed.]

CHAPTER XIV.

CORK—LENTICELS—BARK.

Principal Materials Used.

Normal twigs of *Sambucus nigra* (Elder), still green; also similar twigs becoming, and become, grey; fresh, also in alcohol.
Old twigs of *Cytisus Laburnum;* in alcohol.
Fine bottle corks.
Old twigs of *Ribes rubrum* (Red Currant); preferably fresh.
Potato tuber; fresh.

Principal Reagents Used.

Concentrated solution of chlorophyll, freshly prepared—Solution of alkanet in 50 per cent. alcohol—Fuchsin—Absolute alcohol—Chlorzinc iodine —Potash—Concentrated potash—Chlorate of potash and nitric acid.

We have already, upon various objects, had the opportunity of becoming acquainted with the position and structure of cork,[1] but will once more turn our attention to this tissue, in order to study on the one hand the **Lenticels**, and on the other hand the structure and reactions of the membrane of the **cork cells.**

Cork of Sambucus.—*Cross-sections* through a twig, still green, of *Sambucus nigra* (the Elder) show us, around the broad large-celled pith the separate vascular bundles already connected into a ring by the action of the interfascicular cambium. The cambium ring has also already commenced its activity, and in the vascular bundles, as well as also between them, has formed secondary wood and secondary bast. The primary bast appears supported

[1] Many rapidly-growing stems of woody plants show the development of cork with remarkable beauty. Amongst the best are species of *Hibiscus* and *Abutilon*, very commonly cultivated hot-house perennials. Fresh or in alcohol.—In *Sambucus nigra* it is necessary to avoid the stout succulent shoots which are often met with, and to select only the thin normal growths.—[Ed.]

externally by sclerenchyma fibres. The cortex is from ten to fifteen cells thick. The projecting ridges of the stem show a strong hypodermal sheath of **collenchyma**, which in the furrows is reduced to a layer two or three cells thick. Under the stomata the collenchyma sheath is interrupted by the green cortical parenchyma, which here extends to the epidermis.—In parts of the stem which have begun to appear grey on the surface the formation of the **cork layer** commences, by tangential division of the outermost collenchyma cells immediately subtending the epidermis. The inner of the sister cells thus produced again divides, and it is then the middle cell of the three radially-disposed cells, which further functions as a **cork-cambium** cell. It is easy to recognise, even after the **periderm** has become many layers thick (Fig. 73, *ph*). Outermost in each radial row lies the outer, while innermost lies the inner segment of the original collenchyma cell (*cl*); the flattened cell (*ph*), bounding the inner segment externally, is the **cork cambium** or **phellogen** cell. At first the phellogen gives rise only to cork cells on its outer side; soon, however, it begins, though comparatively but sparely, to cut off cells on its inner side also, which contain chlorophyll grains, and take part in the thickening of the cortex, which the growth in thickness of the stem has stretched. This secondary cortex, developed from the cork cambium, is known as the phellogenic cortex, or **phelloderm.** The whole of the products of the activity of the cork cambium are collected together under the name **periderm.**

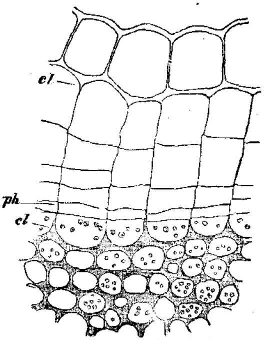


FIG. 73.—Cross-section through the surface of a young stem of *Sambucus nigra*. Epidermis; *ph*, phellogen; *cl* and *cl*, outer and inner parts of the original collenchyma cell (× 240).

Lenticels.—In fortunate cross-sections we can, moreover, determine that the formation of a continuous cork layer is preceded by a peculiar process, which commences under the stomata. The primary cortical cells which surround the air-chamber com-

mence to divide, and the segments encroach laterally upon the neighbouring collenchyma cells. Soon is formed under the stoma a meniscus-like layer of cells in course of division (Fig. 74, *pl*), which produce externally colourless cells, which become rounded (*l*), and internally cortical cells, or **phelloderm** (*pd*). The outer cells are distinguished as **packing cells.** They become brown, but not corky; and moreover, as they increase in number, they soon cause such a pressure on the epidermis that this is torn into a fissure. In this way is produced the cortical pore, or **lenticel.** —If a twig is examined with the naked eye, the lenticel appears as a groove enclosed between two lip-like swellings; the brown colour of the packing cells is easily noticeable. On younger

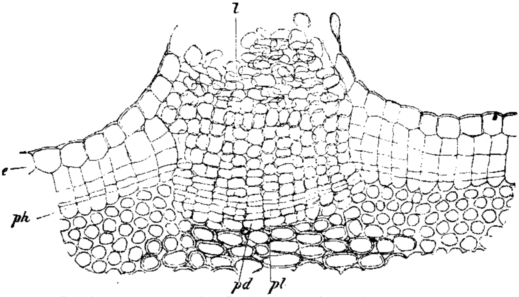


FIG. 74.—Cross-section through a Lenticel of *Sambucus nigra*; *e*, epidermis; *ph*, phellogen; *l*, packing cells; *pl*, cambium of the lenticel; *pd*, phellem (× 90).

parts of the stem the lenticels appear as oval, slightly projecting, spots. Still younger stages are marked out by somewhat brighter colour; and the section must be taken through such places in order to show the earliest stages of development.—Not till after the splitting of the epidermis do divisions begin in the neighbouring collenchyma, which result in the formation of the periderm. The packing cells of the lenticel separate from one another; in proportion as the external cells undergo disorganisation, they are replaced by the action of the cambium. The intercellular spaces of the packing cells are filled with air; by means of them the inner tissue of the stem communicates with the surrounding atmosphere. They replace, therefore, stomata

in the older parts of plants, in which cork-formation has begun; to all intents and purposes they may be looked upon as **cork-stomata.** For the winter, somewhat more compact and resistant packing cells are formed.—*Sambucus* does not show in winter a specially formed closing layer of narrow cells lying close together; while it is met with in many other plants, as also are intermediate layers, which, formed just like the closing layer, are from time to time interposed between the packing cells during the period of vegetation. The cells of these closing and intermediate layers become corky, but have radial intercellular spaces between them, so that they do not bring about complete closure. In older parts of the stem of *Sambucus* the periderm has longitudinal clefts. These pass through the lenticels, without, however, injuring them. The lenticels persist even on quite old stems, while the outer layers of periderm between them scale off.

Structure of the Cork Membrane.—It is advisable to study the structure of the walls of cork cells in *Cytisus Laburnum* (the Laburnum), because here they are somewhat remarkably thickened. *Cross-sections* through the cortex of fairly old twigs show the **periderm** formed of cork cells of one kind only. These cork cells are arranged in regular radial rows. The youngest cork cells are colourless, older yellow, oldest yellow-brown. Those lying at the periphery appear tangentially stretched, often to the obliteration of their cavity. All these cork cells are greatly thickened, especially on their outer side. We can readily distinguish in them, even without the aid of reagents, the delicate **middle-lamella** separating the cells, a strong, distinctly laminate, **secondary thickening layer,** and, within this latter, a **tertiary thickening layer.** Consequently each complete double wall separating two cell-cavities consists of five distinct layers: the middle-lamella, which here represents the primary cell-wall, and is lignified; the two secondary thickening layers, which alone are corky; and the two tertiary thickening layers, which often retain their cellulose character, but here, however, are slightly lignified.

Chemistry of Cork.—If a section of *Cytisus Laburnum* be treated with a freshly-prepared solution of chlorophyll, as concentrated as possible, allowing this to act on the section for some time in the dark, it will be seen that the corky membranes and the cuticle are coloured deep green, while the lignified membrane and the cellulose walls remain uncoloured. This

staining arises from the presence of fatty bodies in the corky and cuticularised membranes. The corky walls and the cuticle agree with oils in so far as to be stained after prolonged action (several hours or more) with a solution of alkanet in 50 per cent. alcohol, albeit not so strongly. Both these reactions enable us to distinguish between corky or cuticularised and lignified membranes, the ordinary relations of which with stains are much alike. If a section is stained in magenta (fuchsin), dissolved in 50 per cent. alcohol, and then washed in absolute alcohol, after a short while the colour is left only in corky and lignified membranes; with longer washing it is left in the cutinised membranes only.

With chlorzinc iodine the cork cells colour yellow to yellow-brown, the younger darker than the older. With potash the cork cells become yellow. The suberised layers of the cell-wall, as well as cuticle, are decomposed if they are warmed in concentrated potash. If heated in chlorate of potash and nitric acid (the maceration mixture) suberised membranes and cuticle give the **Ceric acid** reaction; they form small balls, which melt at 30° or 40° C., and are soluble in boiling alcohol, ether, benzol, chloroform or dilute potash. At ordinary temperatures suberised walls and cuticle energetically resist the action of concentrated chromic acid. Concentrated sulphuric acid, also, remains without recognisable action. They are stained yellow or brown with chlorzinc iodine and also with potassium iodide iodine. Cork material, then, or **suberin** is a fatty body, which consists of glycerine-ester—and other compound esters, together with some infusible constituents which are insoluble in chloroform, and can be decomposed in potash.

After prolonged action of dilute potash the cork layers can be stained with chlorzinc iodine; but the coloration is not due to the presence of cellulose, but rather of **phellonic acid.** While cellulose is wanting in suberised walls, cuticularisation, on the other hand, is due to the supplementary deposit of cutin in cellulose-containing membranes. The cutin of the cuticle and cuticularised thickenings is very nearly allied to suberin, but is not identical with it. Thus phellonic acid which is always present in suberin is not so in cuticle; and identity must not be assumed to go further than is proved by their common constituents. Cutin in general resists the action of potash more strongly than does suberin.

Bottle cork (of *Quercus suber*, the cork-oak,) consists of almost cubical, thin-walled, comparatively large cells; which gradually pass over into somewhat more strongly thickened, flatter cells, marking the limits of the year's production; to which the cubical cells again succeed. Addition of potash solution colours the section yellow, above all the somewhat thicker-walled cells marking the year's limits. Upon these it can now be determined that here also each double wall consists of five layers, just as we found it in *Cytisus*. The reactions for suberin occur here even more beautifully than in *Cytisus*, especially the ceric acid reaction.

Periderm of Ribes.—Often from the phellogen are formed not only centrifugal cork cells, but also centripetal cortical cells, the so-called **Phelloderm**; but usually in much smaller quantity. Rarely does this phelloderm attain such a decided thickness as in the species of *Ribes*. If we prepare cross-sections through older twigs of the Red Currant (*Ribes rubrum*), we find under the thin-walled brown cork layer, first the phellogen, then a thick layer of flat chlorophyll-containing cortical cells. These latter also are arranged in radial rows, which coincide with those of the adjoining cork. In the inner part of the phelloderm the radial arrangement is lost, as a result of tensions acting subsequent to its formation. The innermost phelloderm cells adjoin the collenchyma of the primary cortex. All the structures proceeding from the phellogen are collected under the term **periderm**; in *Ribes*, therefore, the periderm consists of cork (**phellem**) and cork-cortex (**phelloderm**).—Young twigs show that in *Ribes* the origin of the phellogen is somewhat deep in the primary cortex.

Origin and Function of Cork.—While in most cases the cork has origin in the cells immediately hypodermal, in not a few it originates in the epidermis itself, as, *e.g.*, the Willows (*Salix*), and all species of the Pomeae, a section of Rosaceae; or it may even have a pericyclic origin, as on thickening roots. Successive phellogenic layers may be produced, in the stems of dicotyledonous trees, in the cortex, and at various depths in the bast; and the dead tissues external to the innermost layer of cork form the **Bark.**

Cork is also the healing tissue of plants, in that by its means wound surfaces are closed. A phellogen arises in the living cell-tissue under the wound, by which the injured part is soon cut off with cork. The process can be readily followed, experimentally,

upon potato-tubers. We cut a piece from a sound tuber and preserve it in a moderately moist chamber. The cut surface soon takes a clear brown colour, and after a few weeks we can determine that it is covered by a thin layer of cork. Similarly if a cut or scratch be made upon a young twig of most shrubs, or even upon some leaf-stalks (*e.g.*, *Hoya carnosa*), the living cells under those which are wounded will divide tangentially and form a true cork layer. Wound cork may be distinguished as *pathological*.[1]

[1] It seems probable that all cork has, philogenetically, a pathological origin.—[Ed.]

CHAPTER XV.

STRUCTURE OF FOLIAGE AND OF FLORAL LEAVES—TERMINATIONS OF THE VASCULAR BUNDLES—FALL OF THE LEAF.

PRINCIPAL MATERIALS USED.

Leaves of *Ruta graveolens;* fresh; also in alcohol. Or, of *Helleborus niger*, *Syringa vulgaris*, or *Eucalyptus globulus*.

Leaves of *Fagus sylvatica;* fresh, or in alcohol.

Similar leaves, grown in shade and in bright sun, selected for comparison, and placed at once in alcohol.

Petals of *Verbascum nigrum*, or *Papaver Rhœas*, fresh; or *Silene inflata*, *Azalea indica*, *Mimulus luteus;* or standard of Laburnum; fresh, or in alcohol.

Leaves of *Impatiens parviflora;* in absolute alcohol.

Leaf-bases of *Æsculus*, or other plant, prior to fall; in alcohol.

PRINCIPAL REAGENTS USED.

Turpentine and creosote (3 : 1), or Chloral hydrate in water (8 : 5)—Acetic methyl green.

Leaf of Ruta graveolens.—By the aid of a series of selected examples we will now endeavour to make ourselves acquainted with the structure of leaves. We will first deal with foliage leaves, and especially those kinds which exhibit the most profound differentiation in their inner structure. Our first example shall be the garden Rue (*Ruta graveolens*), the leaves of which also usually remain fresh during the winter. The leaves of this plant are bipinnate, the leaflets obovate. Held towards the light, these leaflets show bright spots; these are the internal glands, filled with etherial oil, which are found in the inner tissue of the leaf, and to the oil contained in which the leaf owes its strong smell when bruised.[1] We take in the first place surface views

[1] It is well, however, to bear in mind that such oil-glands, though present in very many kinds of leaves, are not a usual constituent of structure.—[ED.]

of the **epidermis** by means of *surface-sections*. These can be obtained by laying a leaflet flat over the index-finger of the left hand, and held down at its two extreme edges by the thumb and second finger; or upon a piece of bottle cork one portion of the curved surface of which has been cut off to somewhat flatten it. We readily note that the upper side (Fig. 75, *A*) has no, or at the most but few, **stomata**; while these, on the other hand, are numerous on the under side (*B*). Elongated **stomatic pits**, filled with air, lead up to the stomata. Above the glands, as can be determined upon either upper or under epidermis, lie usually four cells (*A*, *sc*), which form the middle of a shallow depression. In thicker parts of the section, where the glands are not opened by

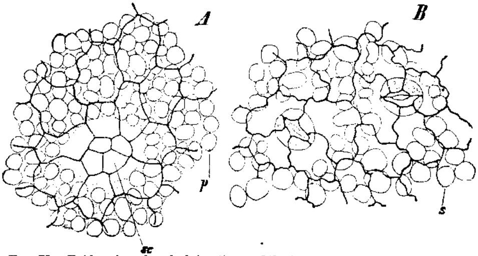


FIG. 75.—Epidermis and underlying tissue of the leaf of *Ruta graveolens*. *A*, epidermis of the upper side; *sc*, epidermal cells over an internal gland; *p*, palisade cells; *B*, epidermis of the under side; *s*, spongy parenchyma. In *A* the intercellular spaces, filled with air, are shaded; in *B* they are left clear (× 240).

the razor, we can see in them a highly refractive yellow drop. With still deeper focussing we can determine that immediately under the epidermis of the upper side lies a green tissue of cells, which appear round in optical section (*A*, *p*). These cells are almost completely separated from one another laterally, and the intercellular spaces are filled with air. Below the under epidermis are situated cells, likewise green and rounded in optical section, but in much smaller number (*B*, *s*). These cells, also, are separated by air-spaces, which, under the stomata, take the form of specially wide air-chambers—the **respiratory, aerating or ventilating chambers.**

After obtaining this general information as to the surface

structure, we proceed to cut *cross-sections;* these we prepare, perpendicularly to the long axis of the leaflet, in the manner already used by us, *viz.*, by placing the leaflet, for the purpose of cutting, between two pieces of elder-pith, or by packing a number of leaflets together for mutual support. The cross-section shows us, occupying the space between the upper and under surface of the leaf, the **mesophyll**, or general internal tissue of the leaf. Proceeding from the upper towards the under side we see first the **epidermis** of the upper side (Fig. 76, *ep'*), then a double or triple layer of parallel chlorophyll-containing cells, elongated perpendicularly to the surface of the leaf, which we call the **palisade cells** or **palisade layers.** We have already proved by the surface-section that these cells are, laterally, more or less separated from one another; on the other hand, the two successive layers are closely attached together by their ends. The elements of the second palisade layer (*pl''*) are somewhat less numerous than those of the first; and two of the outer palisade cells often join on to one of the inner. To these two palisade layers follows a loose tissue, that extends to the epidermis of the under side, and forms a network with wide meshes; this tissue we call **spongy parenchyma;** it contains fewer chlorophyll grains than the palisade tissue. The cells of the upper layer of spongy parenchyma (*sp'*) are closely attached to the inner palisade cells, each one usually joining on to several of the latter cells. None of the palisade cells have their lower or inner ends free; where this appears to be the case (as in some of the palisade cells in the figure), it is simply that the junction does not lie in the plane of the section. So also in the network of the spongy parenchyma,—the cells have no free ends; all the cells are *connected together by their ends. The lowermost layer of* spongy parenchyma (*sp'''*) is elongated in the direction of the lower epidermis, and more or less perpendicularly to it; here, therefore, we have a kind of intermediate formation between spongy parenchyma and true palisade parenchyma. The structure of the leaf in respect of both epidermis and internal tissue is, therefore, manifestly different upon upper and under side, and differentiated in such fashion that we may look upon it as **dorsiventral,** the upper side being considered the dorsal, the under the ventral side. Under the stomata (*st*) can be seen the **air** or **respiratory chambers** (*a*). Single cells in the spongy parenchyma contain **cluster crystals** of oxalate of lime (*k*).

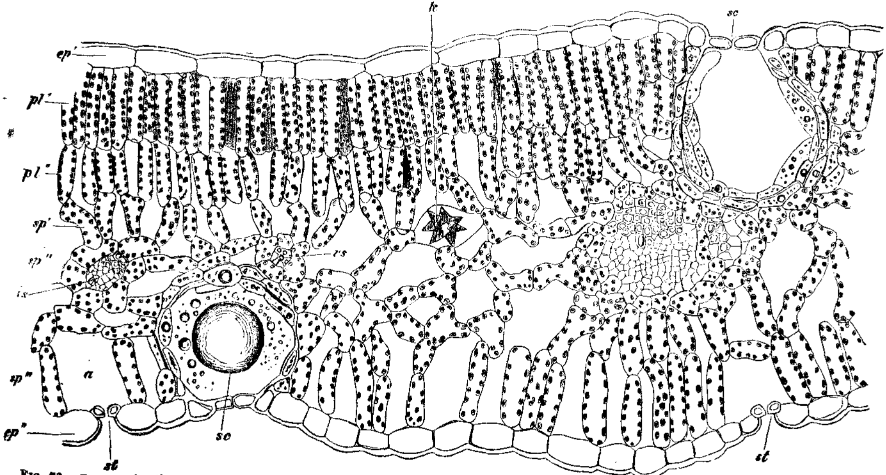


FIG. 76.—Cross-section through the leaf of *Ruta graveolens*. *ep'*, epidermis of the upper side; *ep''*, of the under side; *pl'* and *pl''*, palisade layers; *sp*, spongy parenchyma; *k*, cell containing a cluster crystal (crystallogenous cell); *vs*, vascular bundle; *sc*, *internal gland*; *a*, air-chamber (respiratory chamber); *st*, stomata (× 240).

These cells are devoid of chlorophyll, swollen into a barrel shape, and appear as if suspended between the green cells. At the edges of the leaflets the outer sides of the epidermal cells are strongly thickened. The palisade layer becomes single at the edges, and passes over on the under side of the leaf into the layer of elongated spongy parenchyma (*sp'''*). The **vascular bundles** lie in the spongy parenchyma; the largest, in the **mid-rib** of the leaflet, extend almost from the inner palisade layer to the lowermost layer of elongated spongy parenchyma. In the vascular bundles themselves, we recognise readily the darker-looking xylem, of vascular tracheïdes, and the brighter phloëm. As the vascular bundle bends outwards from the stem, it follows as a matter of course that the xylem portion is upwards, and the phloëm downwards, in the leaf. The radial arrangement of the elements arises from the bilateral, though but temporary, activity of the cambium. Around the bundle is a sheath of parenchyma, the elements of which contain chlorophyll grains, and which join on to the surrounding cells of the spongy parenchyma. The relations of the smaller bundles, of which an example is shown in the figure, are similar. Still smaller vascular bundles (*vs*), which are reduced to a few vascular tracheïdes and bast elements, are also met with in the cross-section. These remain to the last surrounded by a sheath of elongated parenchyma. The **internal glands** (*sc*) impign upon the epidermis of the upper or under side. They are circular in outline, clothed by a layer of thin walled, more or less disorganised cells, indicating their lysigenous origin; to which follows a layer of flat cells with granular contents, and fairly thick white walls. The surrounding chlorophyll-containing mesophyll joins on to these cells. The epidermal cells which lie over the gland are flatter than their neighbours. The volatile oil can be readily removed by alcohol, in which it is freely soluble.

Petiole of Ruta.—Surface-sections taken at the base of the general petiole show the epidermis composed of cells elongated in the direction of length of the petiole, and interrupted alike on the upper and under surface by stomata. Oil glands are also present. Under the epidermis lies a layer of elongated, collenchymatous cells, and then follows the chlorophyll-containing tissue. In *cross-section* the epidermal cells are seen to be more strongly thickened on their outer side; then follows the single layer of thickened collenchyma, absent only under the

stomata. The two or three layers of palisade-like, elongated, green cells are developed to much the same extent all round the stalk, but are looser on the under side. To these follow rounded cells, the outer green, the inner colourless, and becoming larger inwards. In this inner cylinder of colourless cells run the vascular bundles, the strongest in the vertical median plane, and nearer to the under side; the others on either side of the large one, progressively diminishing in size, and each with its xylem portion turned towards the centre of the leaf-stalk. The larger of these vascular bundles are provided on their outer side with strings of sclerenchyma fibres. The activity of the cambium has also apparently lasted longer in these bundles, and it has formed inwardly secondary wood, and outwardly secondary, thin-walled bast.

It is not yet practicable for the student to demonstrate the correlation of the vascular bundles of the phanerogamic leaf with those of the stem; it is sufficient here to note that the former are articulated with the latter in divers ways; that in passing out of the central cylinder or stele of the stem each foliar bundle is accompanied by some of the stelar ground-tissue, and may therefore be considered as a **partial stele** or **schizostele.** The foliar bundle may therefore be looked upon as accompanied by a sheath of ground-tissue (conjunctive parenchyma), which decreases, even as the bundle diminishes in size, with successive branching. Our descriptions have shown, however, that so long as it persists it is quite distinguishable from the mesophyll of the leaf; so that the leaf consists of epidermis, mesophyll and residues of the partial steles. In the ultimate veinlets the mesophyll itself forms the sheath of the partial stele. Traced down through the leaf-stalk the mesophyll joins on to the inner green cortex of the stem. In leaf, as in stem, therefore, the parenchyma may be considered as having two values, stelar (or conjunctive) and extra-stelar (or cortical).

Substitute Leaves.—If *Ruta graveolens* is not accessible, the leaves of the Christmas Rose (*Helleborus niger*), or of the Lilac (*Syringa vulgaris*) can be recommended for study. The *Helleborus* leaves are available throughout the year, and are easy to cut. They show on the upper side a fairly typical palisade parenchyma of broad cells, and below it a loose spongy tissue. *Syringa vulgaris* shows a similar structure, but the leaves are more difficult to cut. Several narrow strips can be cut from a

leaf, all in the same direction, packed together, as already suggested, and cut between elder- or sunflower-pith; in which way not only more but thinner sections can be obtained. Another favourable leaf for cutting, owing to its comparative stiffness, is that of the Australian blue-gum tree (*Eucalyptus globulus*), and this may, with advantage, replace *Ruta*. It is provided with oil glands of a similar character, and the tufted arrangement of the palisade cells is exceedingly well marked.

Leaf of Fagus sylvatica.—As a second object for study we select the leaf of the Beech, *Fagus sylvatica*. The delicate texture of the leaf makes a thin section in this case less easy to obtain, and it will be well to again place straight narrow

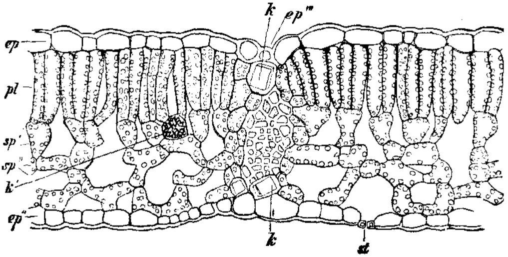


FIG. 77.—Cross-section through the leaf of *Fagus sylvatica*. *ep*, epidermis; *pl*, palisade parenchyma; *sp'* and *sp''*, spongy parenchyma; *sp'* collecting cells; *k*, crystallogenous cells, in *k'*, a cluster-crystal; *st*, stoma (× 360).

strips of the leaf between the two pieces of elder-pith, or, to pack together several of such strips, and then place them between the pieces of pith. Stomata are found on the epidermis of the under side only. Adjoining the epidermis of the upper side (*ep*, Fig. 77) in somewhat fan-like groups of cells, is a layer of elongated palisade cells (*pl*). These palisade cells are again more or less completely separated from one another by intercellular spaces. At their lower ends they bend together into bunches, and to each bunch is joined one or several funnel-shaped, broadened cells of the spongy parenchyma (*sp'*). These latter are connected with the elongated cells of the spongy parenchyma into a loose network, which extends to the epidermis of the under side (*ep''*). Single cells, devoid of chlorophyll, but with a cluster-crystal

(*k'*). are interposed in the spongy parenchyma. The chief veins and the lateral veins of the first order, project strongly from the under surface of the leaf in the form of ribs. This projecting part is about as thick again as the other parts of the leaf. The vascular bundle traverses the projecting rib. This latter is covered with elongated epidermal cells, to which follow elongated collenchymatous cells. To these adjoin cells, each of which contains a simple crystal; and then follows a multilamellar sheath of sclerenchyma fibres, which envelops the whole bundle. On the upper side, the palisade layer is interrupted in a narrow strip over the vascular bundle, and is replaced by collenchyma, above which is a narrow strip of elongated epidermal cells (*cf.* Fig. at *ep'''*). A layer of chlorophyll-containing cells surrounds the sclerenchyma sheath of the vascular bundle, and the cells of the spongy parenchyma join on to these.

Mechanics of the Leaf.—The ribs represent the mechanical system of the leaves, which must be made firm against flexure. The ribs may again be, as far as their mechanical development is concerned, likened to girders. The girders are arranged symmetrically with regard to the surface of the leaf, the plane of the girder being perpendicular to this surface. The upper side of the leaf is "stayed" especially against traction, the under side against compression. The girders in this case are arranged, in each rib in the form of an I, the vascular bundle forming the "filling". The mechanical capacity of the under part of the girder, constructed against compression, is heightened by its removal as deeply as possible out beyond the under surface of the leaf into the projecting rib. By means of the veins the leaf-blade is tightly expanded, and attains thereby the firmness necessary to protect it from tearing.

Smaller vascular bundles, like that of the figure (77), are only protected on the upper and under side by a few sclerenchyma fibres. The ultimate branchlets of the veins are devoid of sclerenchymatous cover, and are invested directly by the sheath of parenchyma. The smaller vascular bundles are accompanied on wood and bast sides by crystallogenous cells (*k*). Above and below them the epidermal cells are somewhat elongated, and form shallow, depressed streaks. From the epidermal cells upon the veins arise long hairs, like sclerenchyma fibres, which are, however, for the most part exfoliated in fully-developed leaves.

Effects of Insolation.—It can be determined without difficulty that the leaves of the Beech have grown especially thick in sunny places, and are so much the thinner in proportion to shade. This increase in thickness, as microscopical investigation shows, affects the palisade parenchyma, which can become very considerably elongated and multilamellar. The palisade parenchyma is indeed a tissue specially adapted for strong light-intensity, while the spongy parenchyma is suited for feebler light. In the palisade cells we see the chlorophyll grains only in profile, *i.e.*, distributed over the elongated side-walls, and therefore, in proportion to the intensity of the illumination, only projecting more or less slightly into the cavity of the cell. In the spongy parenchyma, on the other hand, the chlorophyll grains, according to intensity of illumination, show either surface *or* profile arrangement, *i.e.*, lie parallel to or perpendicular to the upper surface of the leaf. The chlorophyll grains in the palisade layer are first affected by the sun's rays; while the spongy parenchyma only receives light already weakened by absorption in the palisade cells. This disadvantage is partially compensated for by the surface distribution possible in the spongy parenchyma. If, however, the intensity of the illumination is too great for the spongy parenchyma, its chlorophyll grains assume the arrangement in profile. In Beech leaves which are developed in the most intense sunlight, almost the whole green tissue is formed of palisade parenchyma; while the leaves, somewhere about a third their thickness, which have grown in deep shade, have hardly anything but spongy parenchyma.

Physiological Anatomy of the Leaf.—We will introduce a few more physiological conceptions into these morphological studies, and test their accuracy by means of microscopical structure.

In certain coloured **chromatophores**, and, so far as the more highly-organised plants are concerned, exclusively in the green chlorophyll bodies, the assimilation of carbonic acid takes place; that is, these coloured plastids alone are capable, in light of sufficient intensity, of decomposing carbonic acid gas and water, and out of them forming compounds rich in carbon. This process takes place to by far the largest extent in the palisade cells; and from a physiological point of view these cells can be described as, in the highest degree, the **assimilating** or **nourishing cells.** The palisade cells are, as we have already seen, more or less completely separated from one another laterally, and come

together internally into tufts. The products of assimilation, therefore, are not passed laterally from cell to cell, but rather make their way into the interior of the leaf. Here the tufts of palisade cells join on to cells of the spongy parenchyma, which often, at the point of junction, are broadened into funnel form (*sp′*, Figs. 76 and 77), and their function may therefore be that of **receiving** or **collecting cells.** The spongy parenchyma cells which succeed these (*sp″*, Figs. 76 and 77) may, from the same point of view, be designated **conducting cells.** The spongy parenchyma further contains air-cavities, which are in communication with the air-chambers of the stomata; it is, therefore also a **ventilating** or **aerating tissue.** It is also a **transpiration tissue,** since from the surface of its cells specially copious evaporation takes place into the intercellular spaces. Lastly, the collecting and conducting tissue also is, by reason of its chlorophyll contents, an **assimilating tissue.** The spongy parenchyma joins on to the parenchyma sheath of the vascular bundle, to which they ultimately lead the assimilated materials, which are partially conducted in the parenchyma sheath itself, partly in the bast portion of the vascular bundle; hence these last together represent the **conducting strings** or **conducting bundles.** The vascular bundles are, however, at the same time conducting strings for water, which flows in the xylem, from which it is given off to the surrounding tissue, and is also collected in the epidermis, which, in part, functions as a water reservoir. It is the aggregate of this conducting tissue of the parenchyma sheath around the vascular bundle in the form of nerve, or vein-parenchyma,[1] which, together with the strongly thickened "mechanical" cells, promoting firmness, forms the tissue of the projecting ribs of the leaf. The vein-parenchyma is continued into the ground-tissue of the leaf-stalk, which, as we have seen in *Ruta*, is mainly composed of conducting (to or fro) and mechanical elements. In the petiole assimilating cells play only a subordinate part.

[1] It will be seen that three terms, *viz.*, rib, vein and nerve, are used almost indiscriminately by botanical terminologists to represent the same thing. The term *rib* is correct so far as it refers to the mechanical nature of the parts in question, acting just as do the ribs of an umbrella. The term *vein* is correct so far as it refers to the conducting (water and food) function the vascular bundles contained in them. How far the term *nerve* may be *ked upon as correct in its implication must be left to the future to solve;* t, under any circumstances, it must be considered far inferior in appro-teness to either of the other two.—[Ed.]

Structure of a Petal, and Vascular Bundle Ends.—We will now make ourselves acquainted with the inner structure of a petal, and avail ourselves of the favourable opportunity to learn in it the course and endings of the vascular bundles. The petals of the common Mullein, *Verbascum nigrum*, readily permit us to follow the branching of the bundles, and their endings, and to obtain also an insight into the structure of a delicate petal. The air which clings to the bright yellow petal can be easily removed by tapping on the cover-glass. Alcohol should not be used, as it makes the structures indistinct. The petal viewed in water shows a delicate epidermis on the upper and under side, and from two to four layers of spongy parenchyma. Only two layers are found at the edge, from whence the thickness of the petal increases till it attains four layers. The strongest vascular bundles, as well as the finest branches (reduced to spiral tracheïdes only), are invested by a layer of elongated thin-walled parenchyma. This parenchyma sheath closes together over the ends of the bundles. In the cells composing it protoplasmic streaming can be seen. The strongly-branched cells of the spongy parenchyma join on to the elements of this parenchyma sheath. Especially instructive is the view of the endings of the vascular bundles, which show a radiating connection of the cells of the spongy parenchyma with the sheath.

The petals of the common Poppy, *Papaver Rhœas*, can also be studied without further preparation, after the air has been removed by tapping on the cover-glass. Besides the upper and under epidermis, there is here present only one layer of spongy parenchyma. The ends of the vascular bundles are never free; they join, on the contrary, into connected arches at the edges of the leaf. In their entire course they are surrounded by a unilamellar parenchyma sheath. To this the cells of the spongy parenchyma join on from both sides.

The petals of the Bladder Campion, *Silene inflata*, preserved in alcohol, are remarkably transparent. The vascular bundles here hardly form any network; they fork to only a small extent, but the vascular bundle endings can be readily studied. Very good for this purpose are alcohol material of the standard of the flower of Laburnum, and the corolla of *Azalea indica*. The corolla of *Mimulus luteus* is also exceptionally good. Warming in glycerine usually enhances transparency.

Vascular Bundle Endings in Foliage Leaves.—These can be

well studied in most cases of leaves in which the texture is neither leathery nor succulent. We will select for the purpose a Balsam *Impatiens parviflora*,[1] a not uncommon weed of large gardens in many parts of the country. For general information fresh material, when available, will serve; for more careful study of the bundle endings material hardened and decolorised in absolute alcohol is preferable; and suitable sized pieces then laid in a mixture of three parts turpentine and one part creosote, or in a mixture of creosote and alcohol, or in pure carbolic acid, or, and this is best of all, in a solution of eight parts chloral hydrate in five parts water. The leaf soon becomes so transparent that we can obtain any desired optical section of it.—We lay the piece of leaf with the under side uppermost, and see first the epidermis, of very sinuous cells and with stomata; then a very wide meshed spongy parenchyma; then the palisade cells, round in optical section; then the epidermis of the upper side, like that of the under, but devoid of stomata. The palisade layer is very rich in chlorophyll grains, decolorised by the alcohol, while the spongy parenchyma contains but few. Here and there in the spongy parenchyma are found long spindle-shaped cells, each filled with a highly refractive mass of slime, in which a bundle of raphides lies. In young leaves these can be seen in course of development.—The leaf is traversed by a mid-rib, from which proceed stronger and weaker lateral veins. The mid-rib ends at the leaf-apex; the stronger lateral veins of the first order proceed to the edge of the leaf, and there join on in arches to the next higher. From the lateral veins of the first order proceed lateral veins of the second order, and these proceed to successive degrees of branching. The higher the degree of branching the thinner the vein, which ulti-

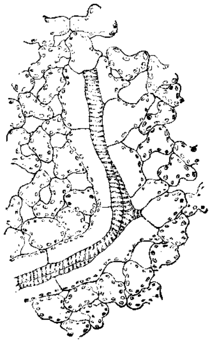


FIG. 78.—Ending of a vascular bundle in *Impatiens parviflora* (× 240).

[1] The transverse section of the petiole of this leaf shows a remarkable gutter-like "starch sheath," which can be followed into every branching vein.—[ED.]

mately becomes so small as even to possess only one annular vascular tracheïde. These thin veinlets lie in the spongy parenchyma, they anastomose with one another into a fine network, and ultimately end blind within the meshes. We can determine that, almost to the last, the vascular tracheïde is accompanied by sac-like elements of the bast; only very short lateral "twigs" consist of vascular tracheïdes alone. Up to the last the bundle also remains clothed by a single layer of mesophyll cells, elongated in the direction of the vein, and devoid of intercellular spaces. This parenchyma sheath also closes over the end of the vascular bundle, quite gapless. It belongs to the mesophyll, and corresponds with the innermost cortical layer in the stem. Up to the last, the tissue of the central cylinder of the stem, which is represented in the leaf by the vascular bundle and the sclerenchyma sheath which usually accompanies it, and the tissue of the primary cortex, which constitutes the mesophyll of the leaf, are separated from one another.

Leaf-fall.—The fall of foliage leaves in autumn results from the interposition of a **separating layer**, or what we may call an **absciss layer**, which is formed earlier or later during the period of vegetation, and which cuts across the articulation of the leaf-stalk. A **periderm** is also usually formed, by which the scar is closed. We will examine the processes a little more closely in *Æsculus Hippocastanum* (the Horse-Chestnut), during the fall of the leaf; and as alcohol material serves just as well as fresh, we can be independent of the time of the year. The absciss layer, as well as the cork layer, lie in the position which is clearly visible externally as the boundary between the brown tissue of the cortex and the green tissue of the leaf-stalk; upwards this boundary strikes the angle which the leaf-stalk forms with the bud in its axis. We cut off the leaf-stalk, with the surrounding parts of the cortex, from the twig, and halve it in a median line. We take now a number of delicate *longitudinal sections* with the razor, taking care that some of them also cut through a vascular bundle. In such longitudinal sections, prepared from fresh material, and examined in water, the cork layer is at once observable, even with a low power, as a clear brownish streak, between the deeper brown cells of the cortex, and those of the leaf-stalk. In alcohol material the cell-walls of the cortex and of the leaf-stalk are colourless. The cork layer is clearly reddish-brown, especially on the cortical side. It consists of six or eight

layers of cells, and joins on to the periderm of the twig with its margins. Its phellogen lies on the side towards the stem. This cork layer is traversed by the vascular bundles of the leaf. Separated by some layers of cells from this periderm, and on the leaf-stalk side of it, the absciss layer, only a few cell-rows thick, passes amongst the roundish cells of the leaf-stalk, recognisable by its yellow colour, the newly-intercalated dividing walls, and the more copious contents of its cells, which likewise contain small starch grains. It is first formed shortly before the fall of the leaf (while the periderm was already present much earlier), and is continued through the living elements of the vascular bundle. Elsewhere, the cells of the leaf-stalk are almost completely emptied of reserve food-materials; they contain, as treatment with iodine shows, mere traces of starch. In the same way starch is wanting, alike in leaf and cortex, within the vascular bundle, although in the cortex it is very abundantly represented in the vicinity of the bundle. The thin-walled elements of the bundle are, on the other hand, filled with highly refractive masses, which give fat and tannin reactions.

If *fresh* sections are examined in water, this latter very quickly shows blue fluorescence, from the **æsculin** which comes out of the stem. Numerous cells of the leaf-stalk contain clusters of crystals, or a single crystal, of oxalate of lime. Preparations treated with acetic methyl-green show in the cells of the leaf-stalk remains of the cytoplasmic sac, nucleus and chlorophyll grains. The yellow grains, into which the chlorophyll bodies break up, give to the leaves their autumn tint.

The fall of the leaf takes place within the absciss layer—the cells of which become rounded, and so disunited; the vascular bundle is torn through in the corresponding part. The leaf-scar is covered by the roundish parenchymatous cells, which lie between the absciss layer and the cork layer, and therefore at first appears greenish. These cells become brown, and dry up quickly in air. At the same time the vessels become filled with brown masses of **wound-mucilage**, which has come from the neighbouring cells into the cavities of the vessels, and plugs them up. Soon the formation of periderm begins in the living elements of the vascular bundle of the scar, only the vessels and sieve-tubes being excluded in the process, and this periderm joins on to that already present, so that the scar is quickly cut off by a complete periderm layer, which continues to develop just as

does the ordinary periderm. Between the cell-rows of this, the flattened and attenuated ends of the vessels can later on be still recognised. The dead ends of the vascular bundles project, to the number usually of 5 or 7, out of the shield-like leaf-scar.

The Bastard Acacia (*Robinia Pseud-Acacia*) serves admirably for the purpose of this study, the processes in which are essentially the same in most Dicotyledons, Gymnosperms, and even in many Monocotyledons. The exact sequence of events, and the time of initiation, however, vary materially. In Ferns, on the other hand, the leaf-scar merely dries.

If strong leaves of *Gymnocladus canadensis*, or of *Ailanthus glandulosa* are laid in a damp, dark chamber, the former in about forty-eight hours, the latter in four days, lose their leaflets on the slightest touch. Longitudinal sections through the leaf base shows that an absciss layer has been formed. Such an absciss layer commences its formation also at the base of the common leaf-stalk at about the sixth or seventh day. Under these conditions, however, a periderm is not formed under the absciss layer. *Fraxinus excelsior* (the Ash) and *Juglans regia* (the Walnut) can also be used in this experiment. The end leaflet of *Juglans* is not exfoliated.

CHAPTER XVI.

THE GROWING APEX[1] OF THE STEM—DIFFERENTIATION OF THE TISSUES—COURSE OF THE VASCULAR BUNDLES.

PRINCIPAL MATERIALS USED.

Buds of *Hippuris vulgaris;* fresh, or in alcohol; or *Elodea canadensis;* or *Myriophyllum.*

Buds of *Euonymus japonicus;* or of the Ash tree, *Fraxinus excelsior;* preferably in alcohol.

Buds of *Equisetum arvense;* fresh, or in alcohol.

PRINCIPAL REAGENTS USED.

Eau-de-Javelle; or concentrated potash and strong acetic acid—Concentrated acetate of potash.

IT will now be our task, by means of carefully-chosen examples, to become acquainted with the structure of the growing apex of plants, and the differentiation of the tissues which takes place there; and, incidentally, to learn something about the route of the vascular bundles through the plant.

Growing Apex of Hippuris.—As the first example we choose a Phanerogam, with a very strongly-developed and easily-prepared "growing point," *viz.*, *Hippuris vulgaris* (the Mare's tail). We take a strong shoot, cut off the end bud about ½ inch under the apex, and first remove from it all the larger leaves. We then hold the bud with the apex downwards, flat between the thumb and index-finger, and endeavour to obtain a *median longitudinal section* of it. For this purpose the razor is passed as perpendicularly as possible between the two digits. First the bud is

[1] Variously known by the terms "growing point," "*punctum vegetationis*," and "vegetative cone". I adopt the term "growing apex" as at once correct, and a suitable complement to the expression "apical growth," in so general use.—[ED.]

thus halved. Each half is cut up subsequently in the same way. Then the section which appears most nearly median is chosen, and in case it does not yet appear thin enough, it is again halved, and so on until a sufficiently thin section is obtained. The operation will at first, perhaps, not be successful, yet in general it presents no insuperable difficulty, and can, at any rate, be attempted: or, instead of between the fingers, we can place the bud between two flat pieces of elder-pith, and draw the razor between these, though in this case the correct cutting of the object is left more to accident. Objects which, like the foregoing, have a certain thickness and firmness, can be also clamped between the ends of two pieces of elder-pith, and cut horizontally, together with the pith, as has been done in earlier cases.[1] From the sections thus prepared in one way or another we select one sufficiently median for examination; we can recognise it by the slender, regularly-formed, growing apex, upon which are formed the leaves in whorls or circles of many members, so that we see them at a short distance from the apex as separate protuberances rising symmetrically from the flanks of the vegetative cone.

The Preparation Microscope.—As in the preparation of the sections fragments of other leaf rudiments which have been cut will frequently obscure the view of the apex, it will be well to clear these away before commencing our study. This can

[1] My own method is as follows: Cut the bud by as near as possible a median cut into two halves; place the halves in water or alcohol, as the case may be. Examine the cut surfaces, and judge by the regularity of the shape which one includes the actual growing apex, or, if it be a large apex, the most central parts of it. Select this half; stick a needle in a holder through it, well outside the median line, at right-angles to the length of the half, so that the cut surface of the half shall lie in a plane parallel with the plane of the needle, and, when upwards, shall have the actual apex to the right hand, or, at least, that side towards which you draw the razor-blade in cutting. With the left hand grasp the needle-holder between thumb and other fingers, but extending the index-finger straight out, and flat, so that the curved side of the half-bud lies on and across the finger, and about a third or half an inch from its end. If the needle be lightly pressed upon the finger, the flesh will yield a little, and the object will sink in and be held somewhat firmly, while at the same time the raised part of the finger beyond the object will serve as a good support for the blade of the razor. Holding the razor-blade as flat as possible, take section after section, quite cleanly, until you consider that you have fully passed the central portion of the bud. If in doubt as to which half-bud contains the actual apex, both halves can be treated in the same way. The proper central section must then be selected under a low power. More than one section may be suitable.—[Ed.]

be done with the aid of a **simple or preparation microscope**—a very useful adjunct to the compound microscope, especially in the preparation of permanent slides. We figure three such simple microscopes, but their number is legion, and form as various as that of the compound microscope itself. An exceedingly simple dissecting stand by Leitz is illustrated in Fig. 79. It has a milk-glass plate as background for the micro-preparation, and an arm to carry lenses. The stand alone costs five marks (shillings). Various lenses can be obtained, ranging from one magnifying 8 diameters and having a working distance of 1 inch, to one magnifying 40 diameters and with a working distance of $\frac{1}{6}$ inch, at ten marks each. Fig. 80 represents a very simple dissecting microscope by Zeiss, over the stage (*ot*) of which is placed a lens (*d*),

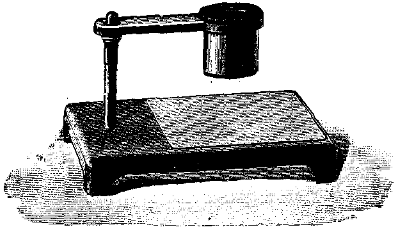


FIG. 79.—Simple dissecting stand by Leitz.

borne on a horizontal arm. The horizontal arm is fixed to a steel upright (*st*), which can be moved up and down inside a tube. By this movement the coarse adjustment is brought about. The fine adjustment is effected on the other hand by turning the screw (*sr*). The instrument is screwed into a dissecting foot, the high ends of which (*p*) serves as resting places for the hands in the processes of preparation or dissection. The instrument is provided with two, or with three lenses, magnifying 15, 30 and 60 diameters, and it is an advantage also to have lenses magnifying five and ten fold.

The larger dissecting microscope of Zeiss (old form) has also a system of lenses (*l*, Fig. 81), consisting of three achromatic lenses which can be combined into an objective (*ob*), a tube, and an achromatic eye-piece. In order to work with slighter magnifica-

tion, the objective can be used alone as a lens, the eye-piece, together with the tube, being unscrewed. The three lenses of the objective can also be unscrewed from one another, and the upper lens alone can be used, the two upper, or the three simultaneously. Magnification of 10, 20 and 30 diameters can be thus obtained. The adjustment is completed by turning the screw head (*sr*).

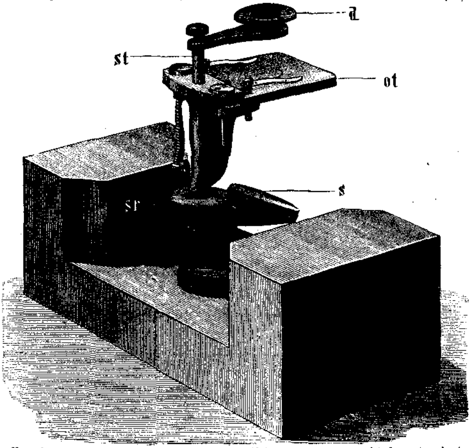


FIG. 80.—Small dissecting microscope of Zeiss, on foot, two-thirds natural size. *ot*, stage; *d*, lens, sheathing toothed support for lens-arm; *sr*, screw for fine adjustment; *s*, mirror; *p*, wooden supports for hands in dissecting, etc.

On both sides of the stage (*ot*) "wings" (*p*) are fixed, to serve as hand supports in dissection.[1]

[1] Many excellent simple microscopes exist suitable for dissection and other purposes, and some are referred to in the Introduction. Essentially each consists of a pillar suspending a mirror, the stage, and an arm to carry the lenses, this arm being movable, preferably by rackwork. It is also of the utmost value that the wrists should be supported to steady the hands in their movements, but this can be effected by loose blocks of wood, etc. An instrument satisfying the requirements of even more than a beginner ought not to cost more than about 30s.

Many simple dissections and other manipulations can however be perfectly well performed by placing the micro-slide, etc., on a suitable back-

The light from the mirror of a simple microscope is often too strong for convenient observation, and it may be an advantage to cover the mirror with a piece of highly-glazed white paper.

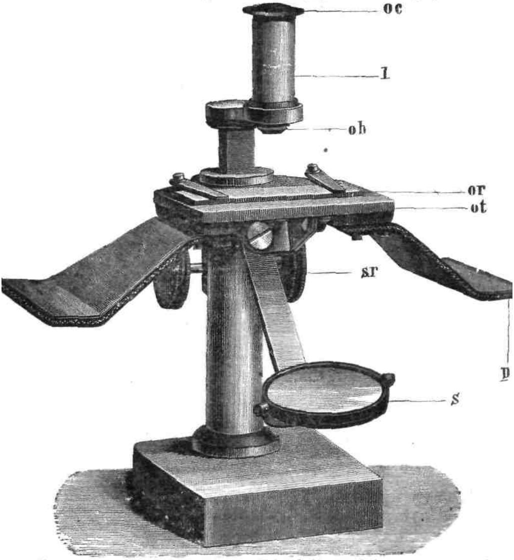


FIG. 81.—Large dissecting microscope (Zeiss), half natural size. *ot*, stage; *p*, wings as arm rests; *sr*, screw-head for adjustment; *l*, system of lenses, of which *ob* is the objective, *oc*, the eye-piece. Upon the stage is an object-slide fixed with the clips.

ground, such as a white or black tile, and fixing in the eye a watchmaker's glass. These can be obtained in cork frames, very light and easy to grasp with the facial muscles; or a good lens may be supported upon a stick set into a weighted foot.

An excellent method of dissection or of manipulation, provided one does not wish to magnify to more than a limited extent, is to have a pair of spectacle frames made, with round hoops, into which can be inserted two of the watchmaker's glasses with aluminium, cork, horn or wood frames. These can be made with lenses of different strengths, and, with a very little practice, perform yeoman's service.—[ED.]

In order to prepare or to dissect with the compound microscope itself, what is called an "erecting eye-piece" can be used in the place of the ordinary eye-piece of the microscope. This "erecting" eye-piece reverses the image of the object; and as, in a compound microscope, the image is normally upside down, it is thus rectified. It is, however, quite possible, though to a beginner very difficult, to dissect, etc., with the ordinary compound microscope. With practice one comes to realise that every movement is reversed, and to govern the movements accordingly. The low powers can then be freely used for dissection and preparation. In dissection, etc., with the compound microscope it is of advantage to have two blocks of wood of suitable size, which can be placed on either side of the stage, and will serve to support the hands.

Whatever instrument is used the method is the same. Place the preparation, uncovered, on the stage of the microscope, and after proper adjustment of the mirror, and focussing, take in each hand a needle fixed in a holder (see Introduction), steady the hands on the rests, bring the points of the needles into the optical axis of the instrument, and endeavour to see both simultaneously in the field of view of the microscope. This will soon be successfully accomplished, and then by means of a few experimental attempts one can learn how to make the necessary slight movements with the needles.

Let us now clean our preparation in this way, and then proceed with its examination. The leaves of *Hippuris* grow in whorls of many members; under the youngest whorl but one the **nodes** of the stem begin to be marked as horizontal denser plates **(diaphragms)** of tissue, above and below which, in the cortex of the stem, proceed the **air-passages.** These air-passages, which extend from one nodal diaphragm to the other, become larger in proportion as the stem enlarges. The **internodes** elongate very rapidly and symmetrically, and in the same proportion their thickness also increases. Under somewhere about the fourth youngest leaf-whorl the formation of vascular tracheïdes in the stem begins. These are very beautifully seen after the addition of a little potash. These tracheïdes traverse the long axis of the stem; they appertain to a **vascular bundle** which grows acropetally, and ends above with individual annular tracheïdes. In about the tenth to the twelfth node the vascular

tracheïdes which appertain to the leaves are first visible. These join those of the stem-bundle. In *Hippuris*, therefore, we have a single central vascular bundle, belonging to the stem only, and therefore a "**cauline**" **bundle**, with which are articulated the vascular bundles appertaining to the leaves—the "**foliar**" **bundles.** In the axils of the leaves, at a short distance from the apex, small flat protuberances begin to be raised, which are the commencement of fan-like scales, each borne upon a short stalk-cell. Only in specimens in course of flower-production do we here meet with the commencing formation of axillary buds; vegetative branching in *Hippuris* is rare.

In order to become better acquainted with the structure of the growing apex, we select a good *median longitudinal section,* and treat it with *Eau de Javelle* (potassium hypochlorite). Gas bubbles soon begin to escape from the preparation. The action must last a shorter or longer time according to circumstances. The most beautiful results are obtained with sections of alcohol material. The *Eau de Javelle* dissolves out the plasmic cell-contents, while the cell-walls stand out sharply; the sequence of cells is then easy to follow. As soon as the necessary degree of transparency is attained, the preparation must be washed with water. If the section has become too transparent, it can be partially restored by treatment with a solution of alum, or with alcohol. If grains of lime, which are separated out, should cling to the preparation, dilute acetic acid should be allowed to run in, in order to remove them. The washed preparation can be preserved in glycerine, but must be first laid in very dilute glycerine, and this concentrated slowly in air. In some cases the preparation is improved by slight staining of the walls with vesuvin, Bismarck brown, or safranin.—In other cases, as in this, *Eau de Javelle* can be used when it is desired to dissolve the cell-contents, and make the cell-walls distinct. Cutinised cell-walls, after some time, are attacked by *Eau de Javelle.* If the cells are very rich in reserve oily materials, or if the structures contain starch in quantity, the sections must lie for a long time, sometimes for days, in the reagent. If *Eau de Javelle* is not at our disposal, then treat the section with concentrated potash solution, wash this out, and lay it in concentrated acetic acid. After some time we examine it either in acetic acid or in acetate of potash. It is an advantage not to place the section direct upon the object-slide, but to lay it upon a cover-glass placed

upon this, and to cover it with a second cover-glass. We can then, if needed, turn over the section together with the cover-glasses, and so examine it on both sides; we must, however, take care that no fluid gets between the under cover-glass and the object-slide. For use in this way we can get thin perforated object-slides, upon which latter the lower cover-glass can first of all be cemented, and thus the whole slide can be inverted.

With pretty strong magnification, we settle, in the first place (compare Fig. 82), that there is a thoroughly definite arrangement of the cells in the "**meristem**" of the growing apex. There are cap-like layers of cells, the separating walls of which form a series of confocal parabolæ. The outermost layer of cells, which covers the growing apex and also extends as a simple cell-layer over the leaf protuberances, is the **initial layer** of the epidermis — the **Dermatogen** (*d*). Under this lie four or more undifferentiated layers of cells (**meristem layers**), which appertain to the **Periblem** (*pr*), and from which the primary cortex of the stem proceeds. Lastly, we find a **central cylinder**, which tapers conically upwards, ending with usually one cell, and out of which, as can be demonstrated lower down in the section, the central cylinder of the stem is formed. This tissue we designate the **Plerome** (*pl*). Epidermis, primary cortex, and central cylinder, have, therefore, in *Hippuris* their own special "**histogens**" or **histogenic layers.**

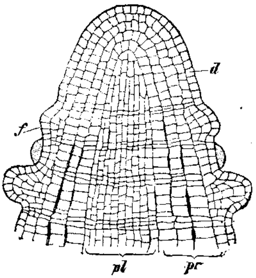


FIG. 82.—Longitudinal section through the growing apex of *Hippuris vulgaris*. *d*, dermatogen; *pr*, periblem; *pl*, plerome; *f*, rudiments of the leaves (× 240).

Not, however, in all growing apices of Phanerogams is the separation of the "histogens" so sharply marked as in this case. In many Gymnosperms (Abietineæ, Cycads) a clear separation between dermatogen and periblem does not exist, and often the periblem is not clearly defined from the plerome. In Angiosperms the dermatogen is always clearly defined, but a clear limit between periblem and plerome is often not present. The question is not therefore in general one of a differentiation of the

tissues which may be traced into the meristem of the growing apex, but rather of the mechanical arrangement of the cell-walls, which give to the young tissues the necessary firmness. Very clearly marked in this arrangement are the rectangular relations of the anticlinal walls, *i.e.*, those running perpendicularly to the surface of the apex, with the periclinal, *i.e.*, those parallel to that surfacé. We can, however, retain the terms dermatogen, periblem and plerome, because the arrangement of the layers of cells which we see in *Hippuris* frequently recurs in the growing apex of Phanerogams, and these terms can therefore be conveniently used for denoting definite regions of the growing apex. From the dermatogen, in fact, amongst Angiosperms, if we exclude the very few exceptions, only the epidermis is derived. The vascular system is, however, not always traceable in its origin to the plerome, but often to the periblem also, as must clearly be the case with cortical leaf-bundles. In the earliest rudiments of leaves we see first, in the outermost layer of the periblem, periclinal divisions set up (at *f*), then follow anticlinal. The dermatogen in the protuberances remains unilamellar; it divides only by anticlinal walls. In the same way in the development of buds, periclinal and anticlinal divisions take place in the outer layer, but in the dermatogen anticlinal only. The vascular bundles which pass out into the flowers and buds must therefore necessarily traverse the periblem.

Substitutes for Hippuris.—Instead of *Hippuris vulgaris* we can use *Elodea canadensis*, which has a very similar, though longer and more slender growing point, which, with great care, can be actually dissected out with needles under the low power of the microscope. Owing to its great length it projects far beyond the rudiments of its young leaves—the leaves which envelop it in the form of a loose bud arising from some distance below the growing apex itself. Species of *Myriophyllum* will also serve.

Bud of Euonymus japonicus.—We will now study a flat-growing apex, such as occurs in most Phanerogams. As an example, we may take *Euonymus japonicus* (the Japanese Spindle tree), cultivated as an ornamental shrub in many gardens, which can be examined at any time of the year, and the buds of which cut very well. We first prepare *cross-sections*, in order to obtain a surface view of the growing apex; and treat the sections just as we did with *Hippuris.* With a low power we recognise the growing apex as a flat hump, surrounded by the youngest leaf-

protuberances. These stand in two-membered, alternating whorls, and are therefore **decussate,** or "opposite decussate," as we are wont to say. Every new pair of leaves starts, after considerable enlargement of the growing apex, in the gaps between the preceding pair of leaves (Fig. 83 *A*). With suitable magnification, it

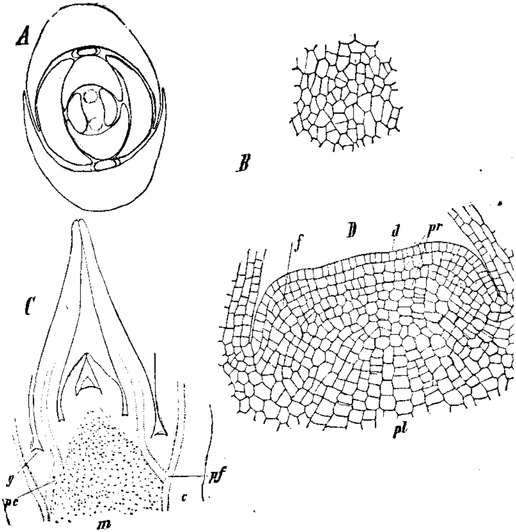


FIG. 83.—Apex of the stem of *Euonymus japonicus*. *A*, apical view of the same (× 12). *B*, apical view of the growing point (× 240). *C*, median longitudinal section through the apex of the stem (× 28). *D*, median longitudinal section through the growing apex (× 240); *d*, dermatogen; *pr*, periblem; *pl*, plerome; *f*, leaf-protuberance; *g*, bud-protuberance; *pf*, leaf-traces; *pc*, procambium ring; *m*, pith; *c*, cortex.

is here easy to follow the arrangement of cells at the apex, as is represented in Fig. 83 *B*. *Cross-sections* taken close under the apex show us first the differentiation of the **procambium,** which will form the vascular bundles. This appears in the section in the form of a rhombic figure, with somewhat projecting and

ounded angles. This rhombic figure appears elongated alternatel in the direction of the newly-entering leaf-traces. The procambium consists of thin-walled, narrow, radially-arranged cells. At the angles of the figure the formation of the elements of the vascular bundles commences; protophloëm elements on the outer, protoxylem on the inner, side of the procambial zone. These regions of commencing differentiation of the elements of the vascular bundles are not delimited towards the rest of the procambium tissue. The procambium zone opens at the places where the foliar bundles enter, in order to admit them. In the axil of each of the young leaves we can see the rudiments of an axillary bud.

A *median longitudinal section*, with a very low power, shows the structure of Fig. 83 *C*. The flat-growing apex, the leaf-rudiments, successively increasing in size, the axillary buds (*g*), the differentiation of the pith (*m*), the procambium zone (*pc*), the vascular bundles, common to leaf and stem (the so-called **leaf-traces**) (*pf*), and the primary cortex (*c*), are to be seen at a glance. Pith and cortex contain large quantities of cluster-crystals of oxalate of lime.—In *fresh sections* examined in water the pith and cortex appear greenish, while the procambium zone appears bright.—In order to follow the arrangement of the cells at the growing apex, we again use clearing reagents. Outermost on the growing apex we find the unilamellar dermatogen (Fig. 83, *D*, *d*); under that three sheath of layers, which we have to designate as periblem (*pr*); and then the massive central cylinder of tissue, the plerome (*pl*), which is not everywhere sharply defined towards the periblem. The growing apex appears very contracted between the two leaf-protuberances last originated; this is its usual appearance. On the other hand you have often to cut for a long time and many buds before you obtain a section which passes through the first leaf-rudiments. If you are successful in this, the form presented is that of Fig. 83 *D*. The growing apex appears then much broader, and the **histogens (or histogenic layers)** can be better traced in it. The formation of the leaves is initiated by periclinal divisions in the *two* outermost layers of periblem (at *f*); the dermatogen remains unilamellar. Exactly the same kind of divisions as for the leaf-rudiments take place in the axils of the third youngest pair of leaves, for the formation of **axillary buds**; they likewise are initiated by periclinal divisions in the hypodermal layers of

cells, the dermatogen dividing only anticlinally, but clearly giving rise to epidermis only, while it is equally easy to see that the cortex arises from the periblem.

In place of *Euonymus* we can use developing shoots of most other trees or shrubs which have decussate leaves. The Ash, *Fraxinus excelsior*, serves admirably.

Growing Apex of Equisetum.—We will now study by means of one of the Vascular Cryptogams a case in which, instead of an **apical meristem**, as above, growth takes place by means of an **apical cell**, from which segments are cut off, to the subsequent division and differentiation of which all the tissues owe their origin. We will select as the most favourable object *Equisetum arvense*, the common horse-tail of cultivated fields. It is comparatively easy in this case to bring the apical cell into view. A preliminary view of the nature of the apical cell, and its relations with the segments cut off from it, may be obtained by a *cross-section* which includes the apical cell itself. This is seen in Fig. 84 *A*. The apical cell has an equilateral triangular base, its sides being somewhat convex, since the three angles tend to be right angles, in accordance with the general rule that a wall, when first formed, tends to form right angles with the walls which it intersects. Parallel with its side-walls are formed new dividing-walls, which cut off flat segments successively from the sides of the apical cell in regular spiral sequence. Each segment subsequently undergoes division by walls at right angles to that first formed. (Fig. 84 *A*).

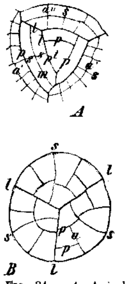


FIG. 84.—*A*, Apical view of the growing apex of *Equisetum arvense*; *B*, optical cross-section of the same, focussed under the apical cell.

For further purposes, shoots in course of development can be studied either fresh or preserved in alcohol. We remove from the apex of the stem a piece about $\frac{1}{4}$ inch long, or rather more, and cut it between the fingers, with the apex downwards, or by the needle method already described. Amongst the *longitudinal sections* produced we look for one which shows the conical growing apex intact. In order to obtain an insight into the arrangement of the cells of this apex, it is usually necessary to make them more transparent. This may once more be effected with *Eau de*

Javelle, or else by the use of a little potash. Should this latter act too strongly, and have "cleared" the growing apex, until its cell-walls become unrecognisable, we can remedy the evil by a suitable addition of water. In fresh sections we must avoid the use of any water-withdrawing medium, as otherwise the growing apex will collapse. Sections of alcohol material can, on the other hand, be laid in glycerine direct, but not after they have been previously placed in water. Sections treated with *Eau de Javelle* cannot be placed at once into concentrated glycerine, but must be

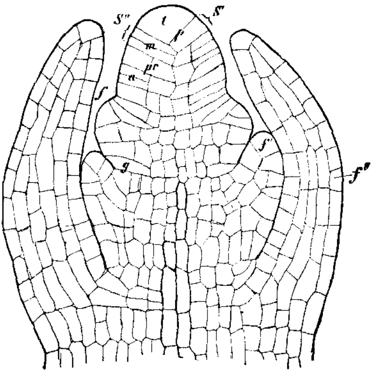


FIG. 85.—Longitudinal section through the growing apex of a main vegetative shoot of *Equisetum arvense*; *t*, apical cell; *S'*, youngest, *S''*, next older segment; *p*, primary wall; *m*, segmenting wall; *pr*, later periclinal; *a*, later anticlinal walls; *f*, first; *f'*, second, *f''*, third whorls of leaves; *g*, initial cell of an axillary bud (× 240).

placed in very dilute glycerine, which is allowed to concentrate by standing in the air. Sections made transparent with potash can be neutralised with acetic acid, and preserved in acetate of potash. As it is of special importance to be able in this case to view the section alternately from its two sides, we lay it, as we did in the case of *Hippuris*, between two cover-glasses.

If the growing apex is cut in the proper direction, it presents its apical cell (a three-sided pyramid upon a convex base), in the form of an obtuse wedge, the apex of which is sunk in the tissue of the growing point, and its base is arched free towards the

exterior. This apical cell divides by means of segmental walls, which are parallel to the existing side-walls of the pyramid, follow one another in spiral sequence, and form segments arranged in three vertical rows. These segments (S) are shown in profile in our Figure 85. They further divide up in definite fashion, and so gradually construct the body of the plant. At some distance from the apical cell, a " bank " is raised upon the growing apex, which grows at its edge with wedge-shaped initial cells Certain parts of this edge, later on, get in advance in their development, and form the free leaf-apices (" teeth ") of what is, lower down, a connate whorl of leaves (" leaf-sheath "). The farther removed from the apical cell, so much the greater are the young leaf-whorls ; simultaneously the differentiation of the inner tissue of the stem progresses, especially the separation into denser, small-celled, thin nodes, and less dense, elongated-celled, long internodes (Fig. 86). First the larger-celled pith begins to separate out in the middle of the stem. In the fifth internode, counted from above (in the figure), the first annular tracheïdes become visible in the procambiun strings, at the outer limits of the pith, and can be traced from here into the next higher commencement of a

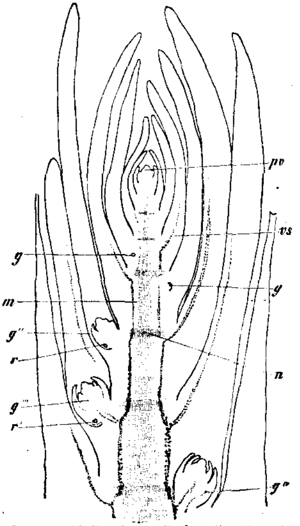


FIG. 86.—Median longitudinal section through a main vegetative shoot of *Equisetum arvense* ; *pv*, growing apex of the main axis ; *g*, initial for a bud ; *g'*, *g''*, *g'''*, *g''''*, stages in the development of such a bud ; *r*, *r'*, the origin of a root on the bud ; *m*, differentiation of the primary pith ; *vs*, spiral vessels making their appearance ; *n*, differentiation of the nodal diaphragms (× 26).

leaf-whorl. Each individual vascular bundle is here common to stem and leaf, and is therefore designated as **leaf-trace.** In each internode just so many vascular bundles run outwards, as leaves are represented in the leaf-whorl. The leaf-traces, lying at first separated from one another, are, in about the node underlying the seventh internode from the apex, connected by side branches, whereby a complete vascular system is formed. Approximately in the tenth internode the pith begins to become hollow, through the breaking apart of its cells. In the node, on the other hand, the pith cells undergo a corresponding augmentation, and remain in union. The lateral buds are initiated by single cells in the axils of the leaf-whorl (leaf-sheath). They stand in whorls, and, as examination of the mature condition shows, alternate in position with the free leaf-teeth of its leaf-sheath, the tissue of which they finally break through at the base, in order to come outside.

CHAPTER XVII.

GROWING APEX OF ROOTS.

Principal Materials Used.

Roots of any *Lemna;* fresh, or in alcohol.
Roots of Barley, or other grass, grown in a pot; fresh.
Roots of *Thuja occidentalis*, or other Conifer; fresh.
Roots of *Pteris cretica*, or other Fern, grown in a pot; fresh.

Principal Reagents Used.

Chloral hydrate in water (8 : 5)—Eau-de-Javelle.

It is desirable to now become acquainted with the **Growing Apex** of Roots (the **Root-tip**). We begin with Angiosperms, which elongate by means of an **apical meristem.**

Root of Lemna.—A general impression can be obtained by means of some species of Duckweed (*Lemna*). Into a drop of water upon an object-slide we place the slender thread-like pendant aquatic root of some selected species of *Lemna*, and examine it with a low power. The root-apex is seen to be covered by an extinguisher-like sheath, somewhat pointed at its forward end. This is the comparatively strongly developed **root-cap.**—A good insight into the structure of the root can be obtained if a fresh root is examined in a drop of solution of chloral hydrate, prepared from eight parts chloral hydrate and five parts distilled water. After some time the root will have become so transparent that we can even study its optical section with a higher power. In some cases alcohol material of *Lemna*, examined in chloral hydrate, performs good service. The extinguisher-like root-cap is only attached to the extreme end of the body of the root; the residue forms a quite free sheath, in the space between which and the root itself, green algæ can often be seen in the fresh

material. In the growing apex the stratified arrangement of the cell layers can be followed, much as in the stem-apex of *Hippuris*. —The root-cap of *Lemna* is not exfoliated, and does not need therefore to be renewed from the interior, a condition which is also shown by numerous other free swimming aquatic plants. In land plants, on the other hand, the root-cap undergoes continuous destruction on its outer side, and must therefore be renewed from the interior.

Root-apex of Barley.—The structure of the root-apex can be studied with comparative ease in the Grasses (Gramineæ), and they are fairly typical. They provide us, it is true, with only one of the possible types of root-growth present amongst Phanerogams, but still one widely spread and instructive, and therefore very suited to give us an insight into the processes in question. In order to obtain favourable material, we choose plants removed with care from flower-pots. If we turn the flower-pot upside down so that the whole contents come out bodily, a result often assisted by jerking the flower-pot slightly down on its rim, the root-apices will be usually found free on the exterior of the ball of earth. For careful study we will select the common Barley, *Hordeum vulgare*, and in order to get general information, first prepare a *cross-section* through an older part of the root. In the middle of the **central vascular cylinder** or **stele**, we find a large duct or vessel, and in its periphery about eight **xylem rays** alternating with the same number of strings of **bast.** As, however, is usual in grasses, the xylem rays extend quite to the **endodermis**, and therefore interrupt the **pericycle.** In the endodermis can be seen clearly the cuticularised strips on the radial walls, showing as dark shadings; outside it comes the thick primary **cortex.**

The *longitudinal section* of the root-apex we prepare between the thumb and forefinger. This can be done well on fresh material only. The section must be sufficiently median; then the structure is plain enough, even without the use of reagents, though here also *Eau de Javelle* can be used with advantage (*cf.* page 210 in the previous chapter). Most prominently can be seen that the body of the root is sharply delimited from the root-cap. We can, in fact, follow a line which is prolonged from the outer surface of the epidermis, continuing over the apex, between the body of the root and the root-cap (Fig. 87). Nevertheless the **dermatogen,** as such, does not pass over the apex, but it can rather be said that the dermatogen (*d*) and the **periblem** (*b*) of the apex come together

in **common initial cells, or histogens.** In our figure only two such common initial cells are present, but there may be several. The dermatogen, as such, can be traced up to these initials; and the periblem also, only one cell thick, impinges upon them.

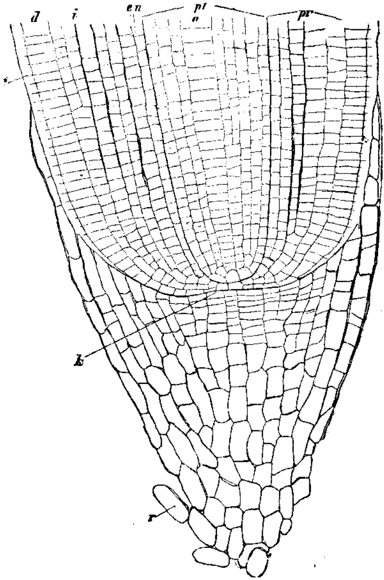


FIG. 87.—Median longitudinal section through the root-apex of *Hordeum vulgare*. *k*, calyptrogen; *c*. thickened outer wall of the epidermis; *d*, dermatogen; *pr*, periblem; *pl*, plerome; *en*, endodermis; *i*, intercellular passage filled with air; *a*, row of cells which will form the central duct; *r*, disorganised cells of the root-cap (× 180).

The **plerome** under this common dermatogen-periblem cap, ends in its own initial cells. Outside the line which separates the root-body from the root-cap are the initial cells for the root-cap, forming a layer of flattened cells which may be designated the **calyptrogen** (*k*). The cells which are given off outwardly from the

calyptrogen are, in virtue of their origin, arranged in straight rows; at first thin, they soon increase in height. At the apex of the root-cap they become rounded; finally separate from one another and become disorganised (r). It is a peculiarity of the Gramineæ that their dermatogen becomes strongly thickened on the outer side (c). This thickened outer wall is glistening, swells strongly, and appears so much the thicker the longer the section lies in water. At the lateral limits of the cells we see highly refractive striæ pass more or less deeply into this thickened outer wall. These are the primary radial walls of the cells; and the older they are, the more deeply they always penetrate into the thickened wall. This wall shows distinct lamination. The **periblem** has rapidly increased the number of its layers by means of periclinal divisions. Between its inner cell layers small intercellular (interstitial) passages filled with air very soon appear, as is represented in our figure by dark lines (*e.g.* at i). The periblem forms the cortex, the innermost layer of which constitutes the endodermis. The **plerome** ends conically in a group of initials; two such initials can be seen in the longitudinal section which we have figured. The plerome forms the central cylinder, or stele. The differentiation of the large central duct or vessel in this central cylinder can be traced up to the group of initials. The cells from which this duct will be constructed are distinguished by their greater breadth (a).

Root-apex of Gymnosperms.—The roots of Gymnosperms show, in many respects, a peculiar organisation in the meristem of their growing apex. We will study more closely *Thuja occidentalis.* The *cross-section* through a fully-developed root resembles that of the root of *Taxus baccata* (the Yew), excepting that the roots of *Thuja* are usually **tetrarch,** *i.e.*, have four primary ligneous rays, or bundles. The *median longitudinal* section through the apex of the root can be examined first in water, and then after treatment with *Eau de Javelle.* It shows a sharply-defined plerome cylinder, which terminates in a few initial cells, and is surrounded by a covering of periblem, some twelve to fourteen cell layers thick. This latter passes over the apex, and forms there its terminal initial layers of eight to ten inner rows of cells, while the outer rows pass over into irregularly arranged, comparatively large cells. These large cells extend to the apex of the root-cap, where they ultimately pass out of union, and become disorganised. The root-cap of

Thuja, and of Gymnosperms generally, consists of the outer layers of the periblem; dermatogen and calyptrogen are wanting. The initial layers of the periblem, passing over the apex of the plerome, divide by periclinal and anticlinal walls. The periclinal divisions increase the number of layers of the periblem, and replace from the interior the elements which are exfoliated from the periphery. The anticlinal walls increase the number of cells in the individual layers, and provide chiefly for the formation of the cortex. As the anticlinal walls in successive layers correspond pretty regularly with one another, they form anticlinal rows of cells, which, straight in the interior, separate from one another externally, like the component rays of spray which collectively constitute the jet issuing from a fountain, forming therefore a constantly-extending series of co-axial parabolæ. The periclinal divisions in the initial layers of the apex have as result that the cell-rows of the cortex, when these are followed towards the point, appear constantly doubled. The most median, straight, anticlinal rows of cells in the periblem of the root-apex are distinguishable from their neighbours. They form a "periblemic column" (Periblemsäule), which is lost in the outermost, brown elements of the root-cap. This column appears clearer, its cells immediately adjoin one another, while those bordering laterally form air-containing intercellular spaces. Moreover, the cells of the column are distinguished by especial richness in starch.—As results from the foregoing relations, the root of *Thuja* can possess no epidermis, the outer surface of the root being composed of the, for the time being, outermost layer of the periblem. If such a layer is followed in the direction of the apex, we shall soon see it pass under another, which now for a time constitutes the surface. The outermost living layers of cells are protected at their surface by the collapsed walls (become brown) of the disorganised layers of cells. The roots of the Gymnosperms have, in general, no root-hairs; we search for such in vain in *Thuja occidentalis*.

The adjoining figure, 88, gives, with low magnification, the structure of a longitudinal section, and will help us to understand it. Naturally, with such a low magnification, the arrangement of the cells can only be indicated. Passing from the exterior towards the interior, we see, therefore, the brown, collapsed covering of cells (x); then the periblem (pr), which can be traced over the apex of the root, and whose outermost layers form the

root-cap; lastly, the plerome (*pl*), the termination of which is not quite clear with a low power. We are inclined to imagine that the upper part of the plerome is bulkier than it really is, because the innermost layers of the periblem, bordering on the plerome, are devoid of intercellular spaces, and therefore (as is shown in the figure) appear just as clear as the plerome cylinder. In the oldest parts of the section the plerome cylinder appears surrounded by a layer of red cells, which, as comparison with the cross-section shows, indicates the endodermis filled with red cell-sap. As we approach the apex these endodermic cells become unrecognisable. Vascular tracheides (*s*) also appear in the older parts of the plerome cylinder. The bright-looking column (*c*) traverses the apex of the periblem. Upon this impinge laterally the air-containing periblemic layers, which extend, however, completely neither to the plerome nor to the surface of the root. The last is composed of large brown cells.

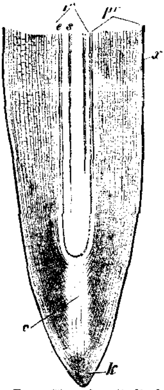


FIG. 88. — Longitudinal section through the root-apex of *Thuja occidentalis*. *x*, outer brown layer of disorganised cells; *pr*, periblem; *pl*, plerome; *e*, endodermis; *s*, spiral vessels; *c*, periblemic column; *k*, root-cap (× 26).

Branching of Roots.—The roots of Coniferæ will serve to make us acquainted with the method of branching of roots in general. In the examination of the roots of *Thuja occidentalis*, it will strike us that they bear their lateral roots in four or in three vertical rows. We readily prove by cross-sections that three rows of lateral roots indicate a triarch, *i.e.*, with three xylem rays, four rows a tetrarch, central cylinder. If we now prepare a *cross-section through a root at the place of insertion of a* lateral root, we shall see that the lateral root projects from one of the xylem rays. Its point of origin, indeed, is in the pericycle, opposite to a protoxylem group, and as the xylem rays run in straight lines in the central cylinder, the arrangement of lateral roots in straight rows is at once accounted for.

Apical Cell of Pteris.—We will now study one more type of root, in which the growing apex elongates by means of **an apical cell.** This apical cell is always a **trilateral pyramid,** and the co-ordination of the segments formed from it is constant. We

study the root of *Pteris cretica* (Fig. 89), but can equally well choose any other species of Fern. By turning upside down a flower-pot in which the fern is growing we easily obtain uninjured root-apices. The roots of *Pteris cretica*, as of ferns generally, are diarch; with the xylem portions alternate flattened strands of bast; the pericycle is unilamellar, the endodermis flattened, the primary cortex has become brown, and in its

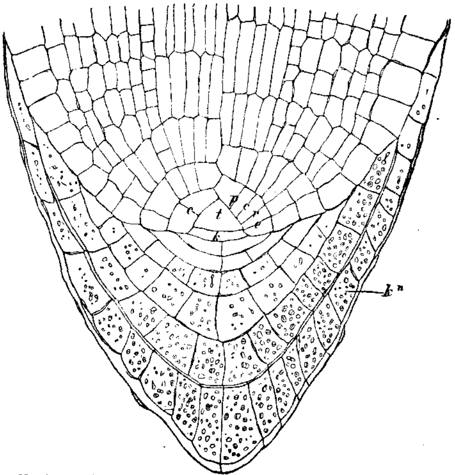


FIG. 89.—Median longitudinal section through the root of *Pteris cretica*. *t*, apical cell; *k*, initial cell of root-cap; k^n, outermost layer of root-cap; *c*, median cortical wall; *p*, outer wall of the central cylinder; *r*, inner cortical wall; *e*, epidermal wall (× 240).

inner part strongly thickened.—We now endeavour to obtain, between thumb and forefinger, a thin, *median longitudinal section* of the root-apex. It is not very difficult to bring into view the apical cell, which is covered by the tissue of the root-cap. This apical cell (*t*, Fig. 89) has, as in the stem of *Equisetum*, the form of a three-sided pyramid, whose convex base is turned towards the cap, while the apex, formed by the junction of the

three side-walls, is sunk in the body of the root. The divisions, as in the stem of *Equisetum,* take place successively parallel to the side-walls, and, therefore, as it were, in spiral sequence; besides these, however, from time to time, usually after each series of three lateral segmentations, a wall parallel to the convex base is formed (compare the figure, at *k*). The apical cell retains its form throughout its divisions; the cell cut off from the base has, however, approximately the form of a segment of a sphere. This cell (*k*) is an initial cell of the **root-cap,** giving rise to one of the layers constituting it. It divides first by a wall perpendicular to its base into two halves; each half repeats this division, so that four approximately similar cells are formed. In these the division is repeated, and always by walls at right angles to the original base, so that an older layer of the cap (k'') consists of a large number of cells. The cells of the older cap layers are full of starch grains. They are gradually disorganised, while the apical cell cuts off continually new initial cells. The outer walls of the, for the time being, outermost cells of the cap, are strongly thickened. The cells which are cut off parallel to the side-walls undergo quite regular segmentation, so that the internal tissues of the root originate from them in very definite fashion. Soon a similar separation of histogens is effected to that present in the root-apex of a phanerogamic root, and gives us the impression as if the apical cell formed only a hiatus in the sheathing of the histogens. The basal cell cut off from the apical cell, giving rise gradually to the root-cap, may conceivably be looked upon as equivalent to the dermatogen; from which point of view the root-cap as a whole is all that represents the epidermis in the root of the fern, and the outer layer, the ostensible epidermis, would be the outermost layer of the cortex, just as the endodermis is its innermost layer.—If in the development of the root we leave out of consideration the root-cap, and the processes which give rise to it, we find processes of growth which in all essential points resemble those existing in the growing apex of a stem. The apical cell of roots, also, does not vary in form as that of a stem does.

If the student finds difficulty in cutting sections of such a thin structure as a root-apex, he may have recourse to the assistance celf embedding, and cutting with a simple microtome such as is co-orcribed in Chapter XVI. In the last chapter of this book will scribed a more complex microtome, by means of which

a series of very thin sections can, after suitable preparation of the material, be readily taken.

Comparison of Stem and Root.—Transition.—A comparative study of the origin of the tissues in the growing apices of stem and root shows resemblance in general plan combined with change in structural detail, especially in respect of the vascular cylinder in the two cases. But as stem and root are continuous, and their tissues also continuous, it follows that somewhere or other there is transition from one to the other. In this transition there is at any rate a twisting of each xylem strand upon its axis, so that the protoxylem is external instead of internal, and a separation of it from its associated bast; but there may also be various grades of branching of the respective xylem and phloëm bundles, so as to increase their number in passing from root to stem; and sometimes branches from adjoining phloëm bundles may come together in intermediately placed pairs, which coalesce, and thus leave the number unchanged though the positions have altered. The character of the epidermis, also, is changed, the exodermis is differentiated, the endodermis becomes more pronounced, and the pith is reduced in quantity. All these changes take place quite gradually, by no means simultaneously, and to very varying extents in different groups of plants. The relations of the two vascular bundle systems to one another show that the central cylinder of roots is not a simple vascular bundle, but that a vascular bundle complex is enclosed in it; but the comparison further shows that the central cylinder of the root corresponds as a whole with the central cylinder of the stem,—a correspondence to which attention was first specifically drawn by Van Tieghem, and which furnished the basis for his "stelar theory," to which attention has more than once been drawn. In both cases the central cylinder is bounded externally by a pericycle, which, however, is, as a rule, much more sharply delimited in roots than in stems, and was thence recognised at a much earlier date under the name of "pericambium". The tissue of the pericycle is especially capable of new developments, and in it various tissues and new structures have their origin. As in roots the cortex is delimited towards the central cylinder by the endodermis, so in stems it is very commonly cut off by a starch-sheath. In some stem parts this delimiting layer has quite the character of a root-endodermis; but it may be entirely wanting, so that the limits of the pericycle towards the cortex are no longer capable of anatomical definition.

CHAPTER XVIII.

VEGETATIVE STRUCTURE OF THE BRYOPHYTA.

Principal Materials Used.

Plants of *Mnium*, or *Bryum*; fresh, or dried, herbarium material, properly softened, will do.
Plants of *Sphagnum acutifolium*, or other species; fresh, or dried plants, properly softened.
Marchantia polymorpha, or other species; fresh.

Principal Reagents Used.

Glycerine-gum—Sulphuric acid.

Stem of Mnium.—Hitherto we have studied only the structure of the stem, root and leaf in **Vascular Plants**; we turn now to the stem and leaves of **Mosses**, from which vessels are absent. We commence with a case in which the differentiation of tissues appears already somewhat advanced; with *Mnium undulatum*. We first of all prepare delicate *cross-sections* through the stem (Fig. 90). In the midst of the stem appears an **axial cylinder**, composed of narrow, thin-walled cells, which we can only conceive as a very simple **conducting bundle** (*t*). Its cells are distinguished from their surroundings by the yellow-brown coloration of their walls. To this conducting bundle adjoin the wider cells of the **cortex**, with greenish-yellow walls, living contents and chlorophyll (*c*). The innermost layer of this cortex is marked by its greater resistance to sulphuric acid, without being in any way sharply distinguishable as a protective sheath or endodermis. At first the cortical cells enlarge somewhat in passing from the interior outwards; at the periphery they become rapidly narrower and thicker walled, and pass over at length, without special limit, into a uni- or bi-lamellar, narrow-cavitied, strongly-thickened **epidermis**. At two or three places, or sometimes only in one, the outer cell layer

of the stem is prolonged outwards into a unilamellar plate of cells, which represents the leaf-wing (f) running outwards from the stem.—*Cross-sections*, which are taken from the lower, leafless, strongly-browned part of the stem, show the walls of the peripheral layers of cells coloured dark-brown. From individual cells of the surface have grown long, brown-walled, repeatedly-branched threads of cells, which are designated **hair-roots** or **rhizoids** (r). These rhizoids have oblique partition-walls. Under many of such partition walls, and always under their side most remote from the base (see below r, left hand) arise branches, which branch again and again in the same way. Only the growing apices of the rhizoids have colourless walls.

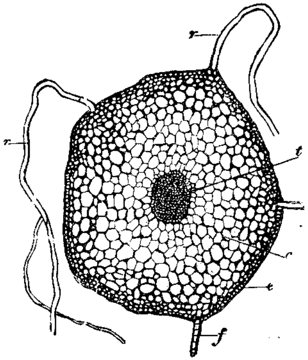


FIG. 90.—Cross-section through stem of *Mnium undulatum*. t, conducting bundle; c, cortex; e, epidermis; f, leaf-wing; r, r, rhizoids (× 90).

The closest similarity with such rhizoids, in respect of branching and the obliqne position of the dividing walls, is shown by the **proembryo** of the typical leaf-bearing mosses, the so-called **protonema**, which is developed from the germinating spore. Its branches, however, so far as they do not penetrate into the soil, are colourless, and contain numerous chlorophyll grains. The **buds**, which develop into moss-stems, are side-branches of this protonema (compare Fig. 91). The close relationship of rhizoids and protonema is shown also in the fact, that rhizoids, if kept damp and exposed to light, can develop a protonema which can give rise to numerous new plantlets. It needs only to lay a turf of *Mnium* with the under side upwards, and to keep it damp, in order to produce numerous green protonema-threads from the rhizoids. The protonema as seen with the naked eye reminds us of tufts of terrestrial *Vaucheria*.

If the cross-section has passed through an injured part of the stem of *Mnium*, the place is seen not to be covered with cork, since this cannot, with a few exceptions, be formed by the Cryptogamia; on the other hand, the walls of the cells bordering the wound are thickened and browned, and so, apart from their broader cavities, simulate the other surface cells.

Near the surface can be seen, in the cross-section, isolated small strings of thin-walled cells, which in coloration also resemble the elements of the central conducting bundle. These are the **foliar conducting bundles,** which have "blind" endings in the

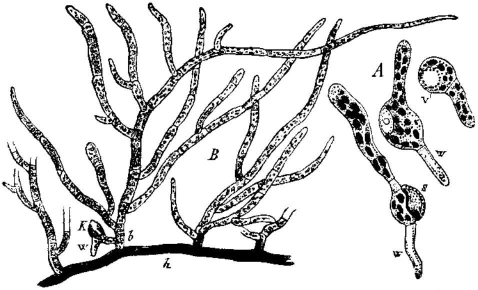


FIG. 91.—A moss, *Funaria hygrometrica*. *A*, germinating spores; *w*, root-hair, or rhizoid; *s*, exospore (× 550). *B*, part of a developed protonema, about three weeks after germinating; *h*, a procumbent primary shoot, with brown wall and oblique septa: from this arise the ascending branches, having limited growth. *K*, rudiment of a leaf-bearing axis with *w*, rhizoid or root-hair (× about 90). (After Prantl.)

cortex of the stem, though in the more fully organised *Polytrichum*[1] they join on to the axial conducting bundle of the stem.

Leaf of Mnium.—A **leaf,** which we examine without preparation in a drop of water on the object-slide, shows a unilamellar lamina, and a multilamellar midrib. This latter ends in a terminal tooth, which consists of a number of rhombic cells. The cells of the midrib are very elongated, the peripheral cells contain chlorophyll grains. The lamina of the leaf is unilamellar; it consists of polygonal chlorophyll containing cells. The seam-like edge of the leaf is formed of elongated, strongly-thickened cells. The outer-

[1] In *Polytrichum*, the inner part of the axial conducting bundle is commonly collenchymatous.—[ED.]

most bear on their edge, at nearly equal distances, one- to two-celled, sharply tapering, teeth. *Cross-sections* through the leaves are obtained at the same time with the cross-sections of the stem. If it is desired to cut cross-sections of leaves by themselves, which, from their small thickness, is no light task, it can be much facilitated if a considerable number of leaves are stuck together with glycerine-gum, and, without waiting for the gum to dry, the object, thus made thicker, is cut between elder-pith.[1] The cross-sections are then laid in water, which at once dissolves out the gum. This method can be used at all times, when it is desired to obtain cross-sections of very thin surfaces. Upon these cross-sections of our moss-leaf, we can determine that the lamina is one-cell thick; the cells at the seam-like margin of the leaf are strongly thickened. The midrib projects more strongly from the under than the upper surface of the leaf. In its midst, somewhat nearer the under side, lies a string of thin-walled cells, in which we again recognise the conducting bundle which we previously saw in the cortex. This thin-walled string is supported on its under side by some strongly-thickened narrow cells.

A withered plant, with the lower cut surface of its stem placed in water, remains withered; but becomes, on the other hand, rapidly turgid if it is immersed with its leaves in water. The absorption of water through the leaves is here, therefore, very active, and that through the stem is very inactive.

Stem of Sphagnum.—The structure of the stem of the Bog-mosses offers special peculiarities. We prepare *cross-sections* of the stem of *Sphagnum acutifolium.* These cross-sections (Fig. 92) show us a broad central cylinder, which in its interior is composed of broad, somewhat collenchymatously-thickened cells; towards the periphery its cells become gradually narrower, and, in the outermost layers, are coloured yellow-brown (*sk*). A special conducting bundle is not present in the interior of this cylinder. It is surrounded by a large-celled outer cortex of usually three layers of cells. The elements of this impinge directly upon the narrow, yellow-brown cells of the inner cylinder. They are distinguished by large round or oval holes (pores) and delicate spiral bands. These pores (*l*) are easy to see, and that they directly join

[1] Good cross-sections of leaves can also be obtained without preparation by cutting through the crowded leaves at the apex (bud) of the stem of an actively-growing moss, such as *Mnium undulatum*, *M. hornum*, or a *Polytrichum*.—[ED.]

together the cavities of these cells can be readily proved in places where the sections have cut through such pores. Not infrequently, moreover, fungal threads are seen in these cells, which pass without hindrance from one cell to the other through the pores. These porous elements of the outer cortex of *Sphagnum* contain only water or air, and are without living cell-contents. To the plant they serve as a **capillary apparatus,** by which the water may be carried to a place of need. The plants are devoid of cuticularised parts; concentrated sulphuric acid immediately dissolves the entire tissue; comparatively the most resistant are the middle-lamellæ,

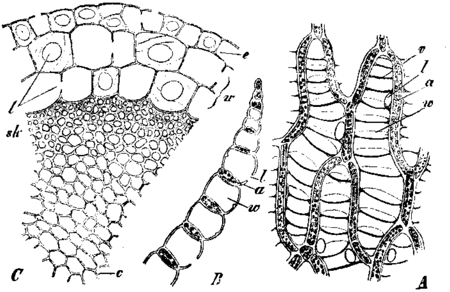


FIG. 92.—*A*, from the leaf of *Sphagnum cymbifolium*. *a*, chlorophyll-containing cells; *w*, water-cells with thickening ribs and openings (*l*); surface view (× 300). *B*, cross-section through the leaf of *Sphagnum fimbriatum*. *C*, part of a cross-section through the stem of *Sphagnum cymbifolium*. *c*, centre; *sk*, sclerenchymatous cortical cells; *w*, water-cells with openings and thickening ridges; *e*, epidermis (× 120).

and their junction "seams," of the yellow-brown outer cells of the central cylinder.

Leaf of Sphagnum.—The leaf expansion is ovate, entire, unilamellar, and consists, as either surface view shows, of two kinds of elements. The one are narrow, chlorophyll-containing (and therefore also with protoplasm and nucleus) living cells; and others are dead, filled with air or water, provided with rings or with spiral bands and intermediate open pores. The fact, which must have repeatedly struck us, that dead air- or water-containing cells, so far as they are not strongly thickened, so often need spiral band, rings or network as thickening of their walls, derives explanation from the circumstance that these cells are devoid of turgidity,

and need this mechanical apparatus in order not to collapse nor be crushed. The green cells of the leaf expansion are all joined together, and form a network with elegantly sinuous walls, each mesh being occupied by one of the dead cells. The green cells serve for the assimilation of carbonic acid gas; the empty cells serve, just as do the corresponding cells of the outer cortex of the stem, as a capillary apparatus for the supply of water. Careful observation shows that the number of pores diminishes towards the edge of the leaf, that they are more prevalent on the under side of the leaf, and occupy the sides of the bulging cell-walls. The edge of the leaf is composed of the narrow green cells, and adjoining these of a single-rowed "seam" of narrower collapsed elements containing water, and slightly thickened on the outer surface. Only the end surfaces of these elements appear to be thickened more strongly, and project outwards proportionally. A midrib is wanting in the leaves, just as is a conducting bundle in the stem; the plants are in this respect, therefore, much more simply constructed than *Mnium;* more complex on the other hand in the formation of a special capillary apparatus.—Cross-sections (Fig. 92 *B*) give us further information as to the relations between the living and dead cells of the leaf, and show us also the pores (*l*) in the latter very clearly.

Thallus of Marchantia.—The *thallus* of *Marchantia polymorpha* (the common Liverwort), is widely spread upon damp ground, and especially in damp green-houses on the surfaces of the flower-pots, and on walls and soil, and is readily recognisable by its rounded or saucer-like **gemmæ-cups**, and perhaps also by its more rarely produced disk-like or umbrella-like **fructifications (receptacles)**. It shows a fairly complex structure. The absence of cormophytic organisation does not therefore necessarily entail simple anatomical structure. The thallus is leathery; it branches by forking (**bifurcation**) of its apex, which lies at the base of an apical depression. If the shoot has forked shortly before, the centre of the previous depression is occupied by a lobe of the thallus, on both sides of which the new apical depressions lie. Along the centre of each shoot projects, on its **ventral**, or under side, an indistinctly delimited midrib. From this proceed outwards and forwards obliquely-directed striæ curving towards the margin of the thallus. At some distance from the apex the thallus is fixed to the substratum by delicate rhizoids springing chiefly from its midrib. If we bring the thallus, with its ventral side turned upwards, under a

simple microscope or a very low power, we can determine, by the aid of needles, the existence of scales (**amphigastria**) which arise from the surface of the thallus. Three different forms of ventral scales are present: **marginal**, which usually extend somewhat over the edge of the thallus, and have become brown; **median**, which lie in the middle line; and **laminar**, which are inserted upon the thallus on both sides of the middle line, but can also be wanting. The median scales, often purple coloured, alternate with one another, and their edges overlap on the midrib. So far as the median or laminar scales, or the former only, extend, arise from the frond fine **rhizoids**, which, covered by the scales, and following their insertion, extend to the midrib, and there run farther forwards in bundles. It is the median and laminar scales which produce the striation on the under side of the thallus, which we have already observed with the naked eye.

If we examine the **dorsal** (upper) side of the thallus with the lens, this appears to be divided into small diamond-shaped areas. *The limits of the areas are dark green, the areas themselves* appear more grey. In the middle of each area a dot-like opening is visible.

We now examine, with stronger magnification, a *surface-section* which is taken parallel to the *dorsal* side of the thallus. We see that the outer cells of the dorsal surface are polygonal, closely united together, and contain numerous large chlorophyll granules. The boundaries of the areas show clearly; each area has its centre occupied by a round opening, which is surrounded usually by four narrow crescent-formed cells containing no chlorophyll (Fig. 93, *A*). Where the section is somewhat thicker, air is seen to be collected under the free outer surface of the area. Into this air space, the **air-chamber**, project chlorophyll-containing threads of cells. The walls bounding the air-chamber laterally are formed of closely-joined cells. These walls are of one to several layers; their cells contain chlorophyll. Individual cells of the surface, and also of the interior, are distinguished by a highly-refractive, irregularly-outlined, clustered body. These bodies in the younger shoots are slightly brownish, in older are coloured brown, contain usually only fat oil, and form the so-called **oil-bodies** of the Liverworts. The cells which contain such a body show no other plastic contents.

Surface-sections taken from the *ventral* side of the thallus show no division into areas. The cells are here more elongated

and poorer in chlorophyll than on the upper side. The **rhizoids** which spring from the ventral surface show an alternative structure. They are either more slender, and provided with peg-like projections into the interior, or thicker and without such projections. Those with the peg-like projections arise out of the frond, as far as the median or laminar scales, or only the former, extend. They lie close to the frond, and follow the mid-rib in bundles, covered by the scales. They probably serve to stiffen the thallus. The ordinary rhizoids proceed chiefly from the midrib, and turn at an acute angle towards the substratum, to which they fix the thallus. All the ventral scales are unilamellar, the median consist of still living cells, the laminar and marginal scales of cells which are already dead.

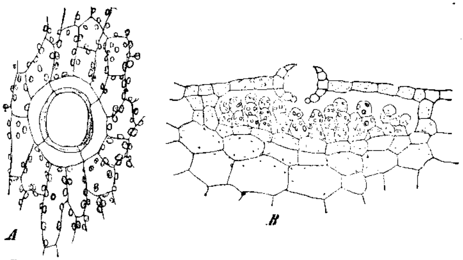


FIG. 93.—*Marchantia polymorpha.* *A*, an air-pore from above. *B*, in cross-section (× 240).

A *cross-section* through the thallus shows us on the dorsal surface first a zone of chlorophyll-containing tissue. The interior of the thallus is composed of broader cells, almost devoid of chlorophyll. In the walls of these cells here and there broad elliptic pits are to be seen. At the ventral surface the two last layers of cells are again narrower, flatter, rich in chlorophyll, and form the so-called ventral cortical layer. Oil-bodies are scattered through the entire tissue. Certain other individual cells are noticeable from their size and highly refractive contents; these are the **mucilage cells.**—A closer study of the outer layers, rich in chlorophyll, of the dorsal surface, completes the conception which we had obtained from the surface view. Outermost we see a single layer of flat cells, which extend free over the air-chamber,

from the walls which bound it laterally. In the midst of the free outer wall is found the **air-pore** (the so-called stoma), which we now see to be surrounded by from about four to eight tiers of cells (Fig. 93, *B*). The pore is narrowed at its upper and under apertures, especially at this latter, and therefore appears barrel-shaped. The cells of the uppermost tier are prolonged into a membranous border. As the air is very tenaciously retained in the air-opening, and the structure is thereby made indistinct, it is *desirable previously to air-pump the air out of the preparation.* Into the air-chamber project from below threads of cells, two or three cells long, and occasionally branched. These threads are especially rich in chlorophyll; they arise from the flat cell layer next below, which is poor in chlorophyll. On the ventral side of the thallus we see on the midrib the lateral, alternating overlapping of the median scales. Between the scales lie the cross-sections of the bundles of rhizoids. *Median longitudinal sections* show the insertion alike of the stronger ordinary rhizoids, turning *off at once from the thallus, and the "pegged" rhizoids overlying* the midrib.

CHAPTER XIX.

VEGETATIVE STRUCTURE OF FUNGI, LICHENS AND ALGÆ—FIXING AND STAINING THE CELL-CONTENTS.

PRINCIPAL MATERIALS USED.

The common Mushroom, *Agaricus campestris;* fresh, or in alcohol.

A Lichen, such as *Parmelia (Anaptychia) ciliaris;* fresh, or dry and soaked.

Fucus vesiculosus, or other species; fresh, and in alcohol; if alcohol material, lay for a few days in alcohol-glycerine.

Nitella;[1] fresh; also for twenty-four hours in 1 per cent. chromic acid. Also *Chara fragilis.*[1]

Cladophora glomerata;[1] fresh; or fixed in 1 per cent. chromic acid, and preserved in camphor-water.

Spirogyra,[1] of some broad-celled species; fresh, and similarly fixed and preserved.

PRINCIPAL REAGENTS USED.

Chlorzinc iodine—Osmic acid—Iodine—Diluted sulphuric acid.—Hæmatoxylin—Picric alcohol—Saturated potash—1 per cent. chromic acid; concentrated picric acid; 1 per cent. chromacetic acid; 1 per cent. chromosmium acetic acid.—P. Mayer's alum-carmine, or para-carmine; P. Mayer's alum-hæmatin—Hæmatoxylin-crystals, and ammonia—Boiled water in quantity—1 per cent. alum—½ to 1 per cent. hydrochloric acid—Aluminium chloride in alcohol; or 5 per cent. acetic acid in alcohol—10 per cent. solution saltpetre, stained with eosin.

Structure of Fungi generally.—The vegetative organs (**thallus**) of the **Fungi**, apart from a number of the simplest forms, consist of elongated, thread-like, more or less copiously-branched elements, the **Hyphæ.** These are either without partition walls (**unseptate**), unicellular throughout their entire body; or, by means of partition walls (septa), segmented into a number of consecutive cells. Moreover, even the most massive fungal structure is composed of such hyphæ, which are then very much interwoven with one

[1] This and many other Algæ, etc., can usually be obtained from T. Bolton, Balsall Heath Road, Birmingham, and Marine Algæ from the Biological Station, Plymouth.—[ED].

another. The hyphæ can indeed, in many cases, come into so close a lateral union, that a tissue is produced, which, as **pseudo-parenchyma**, delusively imitates the appearance of the parenchymatous tissue of the higher plants. This pseudo-parenchyma, however, is a result of the coalescence of cell-threads, and not of cell-division taking place in three planes. In order to study this kind of structure, we take the fructification of a Hymenomycetous fungus. We choose the common Mushroom, *Agaricus* (*Psalliota*) *campestris*, because this fungus can now be obtained at any season of the year, and shows, besides, a comparatively simple structure.

Agaricus campestris.—We prepare first a delicate *longitudinal section* from the stalk (**stipe**) of a fully-developed specimen. We recognise clearly a structure of longitudinally-disposed hyphæ, and can readily tear the section in longitudinal direction with the needles. The hyphæ are directed more or less parallel to one another; occasional ones also run obliquely between the others. Each hypha forms a septate thread, which is branched here and there laterally. The branches arise either close under a septum, or else lower down on the sides. Here and there we come across a "blind" end of a branch. The cells of neighbouring hyphæ not infrequently appear connected by a horizontal branch, and have open communication with one another. In the periphery of the stipe the hyphæ are narrower, and more closely pressed together; just under the surface the walls become brown, and their cavities more or less completely collapse. Towards the middle of the stipe likewise the hyphæ become narrower, but their texture much looser, and, their course quite irregular. Great masses of air here fill up the interspaces between the hyphæ. So long as the destructive influence of water has not made itself felt upon the contents of the hyphæ, very little of these contents is to be noticed; only at the cross-walls they show, here and there, more aggregated. Later on large **vacuoles** begin to form in the cells. Here and there small **crystals** are met with.

The *cross-section* of the stipe has a parenchymatous appearance, which is only lost in the more median parts of the section, where the hyphæ also offer their side views. This pseudo-parenchymatous tissue appears as if composed of unequal, irregular polygonal cells, which leave between them more or less numerous intercellular spaces and gaps (Fig. 94). On careful examination of the section, we notice close in the middle of many cells a

refractive dot (*cf.* the figure). The section has here grazed a cross-wall, and the middle point shows the position of a pit, which is clothed, on either side of the partition wall, with a small aggregation of a highly refractive substance. The cells of the hyphæ contain in the peripheral protoplasm, numerous very small **nuclei**.

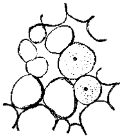


FIG. 94.—*Agaricus campestris.* Part of a cross-section through the stipe. In two hyphæ the section has grazed the cross-walls; a central point can be seen upon them (× 540).

In the membranes of most Fungi **Chitin** has been identified. For its identification the fungus is cut into pieces; these are first treated with dilute potash, then with boiling dilute sulphuric acid, afterwards with alcohol, and finally with ether. The white residue, which upon drying becomes hard and horny, possesses all the properties of chitin. It is insoluble in all reagents excepting concentrated acids.

Lichens—Parmelia ciliaris.—We shall best obtain information about the structure of the thallus of Lichens by means of *Anaptychia* (*Parmelia*) *ciliaris*,—very widely distributed on tree stems; and will confine ourselves in this place to the thallus itself, and not take into consideration the fructifications (apothecia) found upon it. The thallus itself is erect, leaf-like, and shrubby ("foliaceous-fruticose"); on the dorsal (upper) surface, grey-green to bright green; on the ventral (under) surface grey. From the edges of the thallus arise stiff **cilia**, which often become lobed at their ends, and, where they extend to the substratum, adhere to it.—We hold a piece of the thallus between two pieces of elder-pith, and cut *cross-sections* through it. With a sufficiently strong magnification, we see that the thallus consists, on its dorsal surface, of closely interwoven, thick-walled hyphæ. These form the so-called rind, or cortical layer. Passing farther inwards, the curves of the hyphæ separate from one another, in order to form the looser central layer. We can here readily make out that the hyphæ are long sacs, branched from time to time, and divided by cross-walls. At the limits of rind and central tissue lie scattered comparatively large, green, globular cells, the **Gonidia.** They correspond with the Alga, *Cystococcus humicola* (= *Chlorococcum humicolum*). The hyphæ fit closely to the green algal cells, and carry to them crude nutrient sap, for which they receive in return a portion of the substances assimilated in the gonidia. There exists here a **symbiosis**, a conjoint existence of

fungus and alga, which is based upon reciprocal service. At the under surface of the thallus of *Anaptychia* the fungal hyphæ again interlace more closely, so as to form a kind of under rind; or this closer combination does not exist, and the looser central tissue extends to the ventral surface. This latter is in general the case. At the edges of the thallus, however, the rind of the dorsal surface, in all cases, extends underneath to the ventral side. From these edges arise, as we have already seen macroscopically, the fixing cilia (**rhizines**), which now can be made out to consist of closely-combined parallel hyphæ. The walls of these hyphæ have a brownish colour. At their base the threads often fork. — In other lichens the rhizines are apt to spring mostly from the ventral surface of the thallus. Chlorzinc iodine stains the walls of the gonidia immediately a beautiful blue, while the hyphæ only appear yellow to yellow-brown, the so-called **fungal cellulose.**

In *Anaptychia ciliaris* we have a lichen with what is called a layered or **heteromerous thallus,** so called because the algal zone forms a special layer in the thallus. In more lowly organised lichens the thallus is **homoiomerous,** *i.e.*, the gonidia are distributed through the whole tissue. To the last belong also the **gelatinous lichens,** in which the gonidia lie in a translucent jelly, which is traversed by the hyphæ of the fungus. The Algæ, which take part in the formation of the lichen thallus, differ according to their species, are coloured green or blue-green, but belong almost exclusively to the lowest divisions of the Algæ.

Algæ.—Fucus vesiculosus.—The thallus of the Algæ shows a wide range of structural variation, from very simple to relatively complicated. As an example of the latter, we will take the olive-green or brown sea-weed, *Fucus vesiculosus*, known as the "bladder-wrack," and which is so common around our coasts. This *Fucus* is, in its younger parts, flat, leaf-like, traversed by a **midrib** projecting on both sides, and forks more or less regularly in the plane of the leaf-like expansion. It usually bears bladdery swellings, often situated in pairs, one on either side of the midrib, as well as singly at the base of the bifurcations; these bladders are sometimes wanting. In older parts the leaf-like expansion of the thallus is gradually disorganised, and finally is reduced to the much thickened midrib, which has become elliptic in section, and simulates a stem. The stem ends below with a rounded attaching disk, or several stems may arise from a common base. From the

older parts of the thallus, especially from its edges, numerous **adventitious shoots** can often arise. The **growing points** of the thallus lie at the apices of the twigs, in cleft-like depressions, the direction of which corresponds with the plane of the leaf-like expansion, and which can be readily recognised with the lens. Some of the apices may be seen to be double, from equal forking, or dichotomy; but quickly one branch surpasses the other, the latter becomes pressed to one side, and appears as if laterally developed.

For anatomical investigation alcohol material is almost as well suited as when fresh[1]; but fresh material can be sent for great distances without injury, when packed in cases without water. In what follows, therefore, we will concern ourselves preferably with alcohol material, as this permits section-cutting much more readily. In fresh material the swelling of the tissue between the outer and inner layers of the thallus is so strong that it produces great contortion of the sections. The outer layer is in **positive**, the inner in **negative tension**; that is, the outer layers are compressed by the inner layers, and the latter stretched by the outer. Therefore, in the sections, the outer layers lengthen and the inner layers shorten, and the sections twist. Preparations made from fresh material must be examined in sea-water, since they swell strongly in fresh water; alcohol material, on the other hand, we lay a few days previously in a mixture of equal parts alcohol and glycerine, and examine it in the same.

We prepare *surface sections*, parallel to the surface of the thallus, through the midrib and the wings (and, if we wish to see only the primary tissue, at not too great a distance from the growing point), besides *cross* and *longitudinal sections* through different regions. The entire surface of the frond shows, in surface sections, rectangular to polygonal cells, which are arranged more or less clearly in longitudinal rows, often displaced by supplementary divisions. In cross and longitudinal sections these cells appear prismatic, and elongated perpendicularly to the surface. They are clearly distinguishable from the layer of tissue which lies next below, and we will distinguish them as the outer or **epidermoid** layer. The cells of this outer layer are closely filled with olive-coloured **chromatophores.** The chromatophores have the form of rounded grains, or are become polygonal by lateral pressure, and contain the characteristic colouring matter of the Fucaceæ, **phæophyll,** or **phycophæin.** To the outer layer succeed ele-

[1] See note on Alcohol Material of *Fucus* on p. 254.

ments with wide cavities, which in cross-section appear approximately polygonal, but, as the longitudinal section shows, gradually increase in length as we pass farther into the thallus. They contain larger chromatophores, which however are not so crowded as in the outer layer, and therefore can be more readily observed. We include these cells, together with the outer layer, as **cortex.** The cortical cells are connected together by pits with a porous closing membrane. The innermost layer of the cortical cells bears to the midrib the character of a **thickening layer,** which commences its activity at some little distance from the growing point.

By *surface-sections* below the cortex, and by suitable *longitudinal sections*, we determine that the interior of the **midrib** consists of a tissue of longitudinally-elongated cells, running parallel to one another and to the long axis of the frond, and which are connected together into threads. Laterally, these cells communicate by broad pits, and in part also by short prolongations. In the longitudinal direction, the cells of the threads are only separated by delicate partition walls, which are clearly perforated in a sieve-like fashion. A similar structure is shown by the partition walls within the lateral prolongations, and by the closing membranes of the pits. The threads are separated laterally by an intercellular jelly, which has arisen from the swelling of the middle lamellæ and outer thickening layers of the longitudinal walls of the cells. The medulla of the midrib passes over laterally into the "packing tissue" of the two wings, and this appears as a looser network of irregularly-disposed cell-threads, constructed much as in the pith. Here also the partition walls in the cell-threads and the lateral connections are thin, and with sieve-like interruptions, while the longitudinal walls have produced a still more abundant jelly than in the midrib.

Cell-contents and Reactions.—The contents of the cells of the medulla and of the packing tissue are poor in chromatophores, but on the other hand are often rich in highly refractive granules, which cannot be removed by alcohol, but can by ether, which become brown in osmic acid, and therefore are recognised as fat oil. In each cell a nucleus can be made out. Not infrequently the *protoplasmic cell-contents take on a chambered structure* in the water in which we investigate them. With the addition of iodine, the cell-contents, excluding the oil-drops, colour yellow-brown, and the nuclei are usually easily visible; a starch reaction is nowhere found, and the oil here no doubt replaces the missing

starch. The whole distribution of the tissues leads to the conclusion that the processes of assimilation are localised in the cells of the cortex, while the cells of the pith and the packing tissue furnish the conducting system. If we treat the sections with chlorzinc iodine, or with iodine and sulphuric acid, we obtain in either case, but especially with the latter, a blue coloration of the walls. The firmer membrane immediately surrounding the cell-cavity colours deeply, the more distant less so; in the loosest tissue, filling the wings of the thallus, the staining of the jelly tends to be entirely absent. The parts of the membrane around the cell-cavities show distinct lamination. If we allow hæmatoxylin solution to act upon the section, the layers around the cell-cavities stain a deep violet, while the whole jelly takes a bright violet tone and becomes everywhere readily visible.

Growth in Thickness of Fucus.—Even with the naked eye we observed that the thallus in its older parts is reduced to the midrib, expanded into a strong stem. The growth in thickness which brings about the development of this stem out of a midrib takes place in the innermost layers of the cortex. The cells of these layers develop from their lower end sac-like prolongations, which, dividing by cross-walls, and branching from time to time, grow downwards in the jelly between the threads of the pith. Only here and there one of these thickening threads grows into the packing tissue of the wings. *Longitudinal sections* through the neighbourhood where noticeable increase of thickness of the midrib begins, shows us these phenomena without any difficulty. If we examine the *cross-section* of the deeper parts of the stem, we find it constructed internally of sparsely-scattered cells, with wide cavity and brownish contents, and in between these of numerous closely-crowded cells, with narrow cavity and greenish contents. The former are the original threads of the medula, which serve for food conduction; the latter are those which have been intruded in growth in thickness, and have to fulfil mechanical functions. The threads originally present have been pushed apart by those formed later. The base of the stem and the attaching disk are, in fully developed specimens, exclusively composed of these mechanically acting threads. In the outer part of the stem the tissue has also undergone a change. The cells of the outer layer have become brown, are dead, and are gradually cast off. The second layer of the cortex has begun to divide by periclinal walls. We find therefore in the exterior parts of the stem radially-arranged rows

of cells, and this, in stronger stems, to a not inconsiderable degree. The wings of the thallus gradually die up to the midrib, while the growing cortical layer in the periphery of the stem gradually closes together.

The Sterile Conceptacles.—If the thallus is held up to the light and observed with a lens, we notice a number of, as a rule, irregularly-scattered dots, which are wanting only over the midrib. Even with the naked eye these dots appear as protuberances. On surface-sections each appears as a round opening, surrounded by a projecting rim, and out of which a tuft of long hairs projects. They are pits, no doubt allied to the conceptacles which we shall study hereafter in considering the sexual processes, and which we can call **sterile conceptacles.**—If we prepare a *cross or longitudinal section* through such, it appears as a flask-shaped hollow. The hollow is surrounded by the cells of the inner cortex. From the cells at the base of the hollow arise long threads, composed of elongated cells, which project from the mouth of the sterile conceptacle. It is quite possible that these hairs facilitate the absorption of nutrient materials from the surrounding water. In sections through older parts of the thallus, we can find, between the long hairs in the pits, also bundles of shorter unicellular hairs, which do not reach to the mouth of the pit. If, lastly, still older parts are examined, where the pits present themselves as brown spots, we find the outer parts of the long hairs destroyed, and the opening of the pits closed by the basal parts of these hairs, by the short hairs, and by a brownish mucilage.

The Bladders.—Sections which are taken through a young **bladder** or **vesicle** of the thallus, show its interior filled with a plexus of the same threads which we found in the wings of the thallus. Scattered in the jelly between the threads are bubbles of gas, which sometimes tear the loose tissue and form large air chambers. The older bladders are quite hollow, filled with air remnants of the threads of cells being found on the wall. This latter is covered by the outer layer, and shows a thick cortex arisen from the tangential division of the other cortical cells.

The Characeæ.—Nitella.—The small but widely-distributed family of the **Characeæ** (or Stoneworts) occupies a well-nigh isolated position in the Vegetable System, but can best be included amongst the green Algæ. They are characterised by a remarkable vegetative structure, and, as we shall see hereafter

by a still more remarkable structure of the sexual organs. They are found not at all uncommonly growing at the bottom of ponds and streams, and in bog holes, forming long dense tufts of green thread-like stems, rooted in the mud by means of delicate hairs, or **rhizoids.** The Stoneworts are divided into two genera, *Nitella* and *Chara*, distinguished for our present purpose by differences in the structure of the stem.—As the more simply organised, we may commence our study with *Nitella*. The slender axis, examined with the naked eye, is seen to be segmented; long bare segments alternating with whorls of appendages. The former are the **internodes,** the latter arise from the **nodes.** We have here therefore an apparent approach to the condition of affairs met with in the flowering plants already studied. Each internode consists of a single very elongated cylindrical cell. The principal characteristics of this **internodal cell** we have already examined (see p. 50). It possesses a firm, well-developed, transparent cell-wall; underlying this is a lining layer of protoplasm, in which are embedded innumerable regularly-arranged chlorophyll bodies, with thin layers of protoplasm between them. Inside the stationary layer is a layer of protoplasm in the active streaming movement known as **rotation.** The general cavity of the cell is filled with cell-sap. The **neutral lines** will be readily visible; these, and the lines of chlorophyll bodies, pass spirally along the cell. The **node** consists of a single disk-like cell, on the edges of which are borne appendages ("leaves," or "branches"), likewise consisting each of a thread of cells. From the lowermost nodes arise long, often branched **rhizoids,** divided by oblique partition walls. As we pass higher up the stem the internodes become progressively shorter, and the nodes and their appendages therefore nearer together, until at the summit they are crowded together into a terminal **bud.**

Dissect out this **bud** under the microscope by means of needles. This is best done from material laid for from twelve to twenty-four hours in a 1 per cent. watery solution of chromic acid. The bud is laid upon a slide, and with needles the lowest portions are successively removed so long as it appears safe to do so. These portions are then removed out of the way, a small drop of glycerine added to the specimen, the cover-glass laid on, and by gentle pressure with the needles upon the cover-glass, while observing through the microscope, the bud slightly crushed. It is probable that the bud will open, and disclose its structure. At the apex

is seen a single hemispherical cell, the **apical cell**, in which is visible a well-defined protoplasmic body, and a rather irregularly-shaped nucleus. In some cases two nuclei will be seen, placed one nearer the flat side of the cell, and the other nearer the free apex; such a cell is about to divide. The dividing wall is formed parallel to the flat basal wall of the apical cell. In this way cells are cut off from the apical cell. The cell which resulted from the previous division will be seen adjoining the basal wall. Tracing back from the apical cell, it will be seen that the cells become alternately nodal and internodal cells.

Chara.—Let us now turn to *Chara*, selecting for the purpose the widely spread *C. fragilis.* Externally this plant resembles *Nitella*, but it will be seen upon examination that the internodal cells, instead of being naked, are covered with a layer of cells which we call the **cortex**, consisting of slender cells which are themselves divided into cell-rows. Similarly, the nodes are flat disks of cells, of which the external ones give rise to the whorls of appendages, and also to the cortical cells. Rhizoids arise from the lower nodes, as in *Nitella.* The leaves are segmented like the stem, and from their lower nodes produce leaflets. The terminal cell of the leaf has no cortex, and in it the general features of the internodal cell of *Nitella* are repeated. The bud resembles that of *Nitella*, excepting that the **nodal cells** undergo early segmentation by vertical walls, so as to produce a disk consisting of a central and a layer of external cells, these latter growing out in such fashion as to overlap the undivided internodal cells, and, by subsequent division, produce the cortex.

Cladophora glomerata.—The genus *Cladophora* consists of abundantly-branched green threads, whose cells decrease in thickness with the successive branchings. These are the most widely distributed of all fresh-water algæ, and any species is suited for examination. The determination of species is, however, very uncertain in this genus. We select the dark-green, undulating, tufted *Cladophora glomerata* for examination. This is corymbosely branched, the branches arising, as in all other Cladophoreæ, from the upper end of the cells. The branching proceeds acropetally, so that the end segments of the branches act as apical cells. Subsequently branches arise also from the older segments, producing what we may call **adventitious shoots.** With sufficiently strong magnification, the green peripheral cytoplasmic layer of the cells is seen to be composed of small polygonal plates, the

chromatophores (Fig. 95, *ch*), separated by delicate colourless lines. In each plate more or less numerous pale grains (*a*) can be seen ; beside these lie comparatively large, more or less regularly spherical bodies, more strongly refractive, which are known as **pyrenoids** (*p*), which have a protein reaction. Each pyrenoid is surrounded by a sheath of **starch.** The cells are seen to be filled internally with cell-sap, which is traversed by colourless, exceedingly thin **cytoplasmic plates,** which, proceeding from the lining layer, divide the cell cavity into irregular, unequal, polygonal chambers. Here and there chromatophores can be seen in the inner protoplasmic plates. By focussing so as to get an optical section, it will be seen that colourless protoplasmic spheres here and there project from the lining layer into the general vacuole of the cell-sap. These are the nuclei, and in each, in especially favourable situations, a nucleolus can also be distinguished. In *Cladophora*, as is clearly proved by this examination, we have therefore **multinuclear cells,** or the whole thallus may be said to be **non-cellular.**—If now the preparation is pretty strongly crushed, we see in the flattened cells, the contents of which are somewhat withdrawn from the walls, the individual chlorophyll plates, separated from one another, and rounded. At the same time the small grains and pyrenoids show up clearly in the chromatophores, which now appear, like the chlorophyll grains of higher plants, acted upon by the water. If we now add a little iodine solution to the preparation, the small grains, and also the outer sheath of the pyrenoids, colour violet, and are thus proved to be starch ; in the green chromatophores, however, they appear brown, and the nuclei, visible here and there, also take a brown colour.—We must not omit in this preparation to search for uninjured cells, in which starch grains and amylum

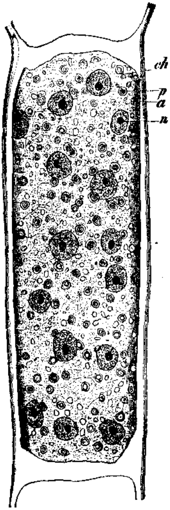


FIG. 95. — *Cladophora glomerata.* A cell of a thread from a chromic-carmine preparation. *n*, a nucleus ; *ch*, chromatophores ; *p*, pyrenoids ; *a*, starch grains (× 540).

bodies are stained in their natural position, and are very well defined; and we can also, by deeper focussing, distinguish the nuclei.

Reactions for Chlorophyll.—We now examine another thread, which we lay direct into a drop of picric alcohol, when the pyrenoids are sharply defined in the yellowish brown stained cell-contents. After a short time irregular brown bodies appear in the chlorophyll plates, which proceed from the disorganised chlorophyll, and give us the **hypochlorin** or **chlorophyllan** reaction. The same reaction will be obtained under the influence of other acids. The chlorophyllan reaction may be taken as characteristic of chlorophyll. Another reaction, likewise characteristic, is that which arises upon treating chlorophyll with a saturated solution of potash in water. The chlorophyll bodies are almost immediately coloured yellow-brown, and, after at the most $\frac{1}{4}$ to $\frac{1}{2}$ hour, again become green. The conversion of the yellow-brown into the green colour takes place immediately upon warming to boiling, or upon addition of water, somewhat less rapidly upon addition of alcohol, ether, or glycerine.

Fixing and Staining Methods.—In order to be able to study the **nuclei** more closely, and to obtain a complete insight into their distribution, we will bring other methods into use. This will, besides, give us opportunity of learning some approved methods of "fixing" and staining, which histological studies have, in recent times, to thank for not unimportant advances. We place some branches of the *Cladophora* in 1 per cent. chromic acid, other small portions in concentrated picric acid, others in 1 per cent. chrom-acetic acid (chromic acid 0·7 per cent., acetic acid 0·3 per cent.), and still others in chromosmium acetic acid (chromic acid 0·5 per cent., osmic acid 0·2 per cent., glacial acetic acid 0·2 per cent.). In doing this, we must take care that the reagent is at least 100 times the bulk of the object to be fixed. The chromic acid, and the chrom-acetic acid, we allow to act for some hours, even without disadvantage for twenty-four hours, the picric acid for about twenty-four hours, or the chromosmium acetic acid for about half an hour only. All these preparations must afterwards be washed most carefully in running water; they can, with advantage, remain for up to twenty-four hours in water which is frequently changed. Specially careful washing is required to remove picric acid from the preparations when they have to be stained with hæmatin-ammonia. The variously "fixed" and

well-washed preparations are now laid in watch-glasses with P. Mayer's alum-carmine, or P. Mayer's para-carmine. The material should lie in the carmine stain for several hours. Another portion of the threads can be stained in P. Mayer's alum-hæmatin. It is best from time to time to control the depth of the staining by slight examination under the microscope, and to take the material out when it has stained sufficiently. This is perhaps best effected if, instead of staining in a watch-glass, the process is carried on upon an object-slide, in which a round or oval hollow has been ground. The slide can be placed bodily on the stage of the microscope, and is handier in use for this than a watch-glass. If in spite of this care the object should be *overstained*, that is, has been stained too deeply, it should be laid in pure water, or in about 1 per cent. watery alum solution, and left in till the depth of the stain has been reduced so far as needed.—In order to be able to stain a picric preparation according to the ammonia-hæmatin method, we must have previously removed from it every trace of picric acid. For this purpose we transfer it to a comparatively large quantity of boiled water, which we repeatedly change. In this water, freed from carbonic acid gas by its previous boiling, the object remains for twenty-four hours, after which it can be stained. For this purpose we put some crystals of hæmatoxylin in a small quantity of distilled water, and aerate it with ammonia gas. This latter we effect with the aid of a wash-bottle containing some ammonia solution, in which the two glass tubes do not reach the fluid. The hæmatoxylin crystals now dissolve with a beautiful violet colour. The solution is greatly diluted with distilled water, and the preparation allowed to lie in it for some hours. The exact time for coloration can here also be directly controlled. The preparation is, with advantage, somewhat overstained, and afterwards steeped for several hours in distilled water. This method of staining is somewhat troublesome, but often gives, however, the most exquisite results. Preparations hardened otherwise than with picric acid are little suited for staining with ammonia-hæmatin. The preparations treated with alum-carmine are also washed with distilled water. If the protoplasm remains somewhat stained, the stain can be removed by 1 per cent. alum solution or ½ to 1 per cent. solution of hydrochloric acid. If the staining has been with para-carmine the washing can be with a suitably weak solution of aluminium chloride in alcohol, and, if this does not suffice, with alcohol containing 5 per cent. of acetic acid

Mounting.—If, after our study of the preparations is completed, we desire to make *permanent preparations* from them, we can select for a mountant either glycerine or glycerine-jelly. For carmine preparations 1 per cent. acetic acid may with advantage be added to the mounting medium. The preparations in question must not be transferred direct to the mounting medium, since the cells, as the result of sudden withdrawal of water, would collapse. They are, therefore, first laid in very dilute glycerine, which, by standing exposed to air, very slowly concentrates. The threads can then, without prejudicial results, be transferred to glycerine, or to glycerine-jelly. The glycerine preparations are closed with Canada balsam. The glycerine-jelly preparations need, for a while at least, no further enclosing.

We will now submit the various fixed and stained preparations to close study, and find that the chromic acid, or chromic acid mixture preparations, stained with forms of carmine, on the one hand, and preparations which are suitably fixed and stained with alum-hæmatoxylin and ammonia-hæmatin on the other hand, prove to be the best. It must, however, be explicitly stated that this result refers only to the objects in question, and for other objects some other method, which here is less advantageous, might have the preference. It also happens, only too frequently, that a stain formerly approved fails for unknown reasons, and a conclusion should therefore never be based upon an isolated case. Fixing and staining of the cell-contents has indeed become a special art, which must he learnt, and requires much practice, so that in our first attempts we must be prepared for failures. We have chosen *Cladophora* as a suitable object for introduction to the various methods of hardening and staining; whoever wishes to limit himself to the surest and most reliable method, will harden in the above way in 1 per cent. chromic acid, and afterwards stain, one portion with either of the recommended carmines, another with alum-hæmatoxylin. The carmine stains almost always succeed.

In the carmine preparation (Fig. 95) the **nuclei** stand out quite sharply. The **pyrenoids**, together with the rest of the protoplasm, remain almost unstained, and the starch-grains also take no colour. The nuclei, to which we specially turn our attention, are distributed pretty uniformly in the cell; they lie on the inner side of the chlorophyll layer, and project into the cavity of the cell. Each nucleus shows a more darkly-stained

nucleolus, and appears, besides, as if finely granular or finely porous. The alum-hæmatoxylin or hæmatin preparations show the nuclei stained dark, and likewise, though more faintly, the pyrenoids. The starch grains are not stained, but on the other hand the microsomata (microsomes) of the cytoplasm are, and almost as darkly as the pyrenoids.

Spirogyra. Cultivation of fresh-water Algæ.—The genus *Spirogyra* furnishes us with a simple filament or thread of cells. We choose for examination a species which has a central, readily visible nucleus. So constituted, for example, is *Spirogyra majuscula* (*S. orthospira*), which is met with now and then, not exactly rarely, but sporadically, in pools; but other species with a central nucleus will serve equally well for examination, and will differ but slightly in the essential relations of their structure. If once in possession of good *Spirogyra* material you should endeavour to keep it in cultivation. In room cultures *Spirogyra* is readily spoilt. It is best to keep it in a large vessel, filled with rain or tap-water, in the window of a cool north room. It must be quite quiet, and left to itself. For the continuous culture of most fresh-water algæ it is advisable to use a large aquarium, holding from thirty to forty litres of water. Such an aquarium is made of glass walls in a metal rim. In the middle of the bottom are fixed two upright tubes, of which one is connected with the water supply, the other serves as an overflow. With the former a bent movable tube is connected, so that the entering jet of water can be directed to different parts of the aquarium. The mouth of this tube should be situated about 10 cm. under the surface of the water, which latter is determined by the opening of the exit tube; and this is provided with a suitable cover in order that free swimming algæ may not be carried down it by the flowing water. It is generally well if the algæ in such an aquarium are fixed in some way, whether by stones, or by individual threads being drawn up on the glass sides so as to dry upon them. In such aquaria the algæ are not only kept provided with fresh water, but also subjected to a desirable cool temperature. The direct action of solar rays should always be avoided.

For special purposes it may be desired to cultivate algæ in a nutrient solution. For this, a solution known as "Knop's" may be specially recommended. It consists of calcium nitrate 4 parts, magnesium sulphate 1 part, potassium nitrate 1 part, potassium phosphate 1 part. In preparing it, we first dissolve the two

potassium salts and the magnesia, and after suitable dilution add to it the separately-dissolved calcium nitrate. Under these circumstances only a very small portion of insoluble calcium phosphate is separated out. It is not as a rule necessary to add iron, since the traces which come into the culture fluid with the material, and the rain- or tap- water used, will generally suffice. This nutrient solution is used for the culture in such proportion that the culture water contains 0·2 to 0·5 per cent. of the salts. If the weights given above are taken as grains, the amounts in question would suffice for about two, or if as grams for thirty, litres of water.

Many fresh-water algæ, *e.g.*, *Ulothrix*, from rapidly-flowing water cannot be cultivated for long in aquaria, but can be kept in a ruuning spring or fountain, if they are placed in the water while still attached to the stones upon which they were growing.

The cells of *Spirogyra majuscula*, when fully developed, are about one and half to twice as long as thick (Fig. 96). The cell-wall is lined by a delicate, colourless, lining layer of protoplasm, which becomes clearly visible if the cells are **plasmolysed**, *i.e.*, if the protoplasmic body of the cell is made to contract by some water-withdrawing medium, such as sugar solution, glycerine, solution of common salt, or of saltpetre. To the colourless lining layer follow eight to ten **chlorophyll bands**, which usually appear pretty steep and closely wound. The bands have a finely-undulating outline, and are transparent enough to admit of a view into the interior of the cell. At irregular distances in the bands are embedded denser, globular, colourless, bodies—the **pyrenoids** with which we have already become acquainted. These pyrenoids are ensheathed in a hollow sphere of small starch grains. With iodine solution they

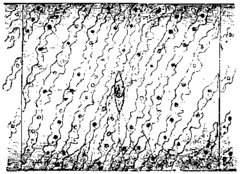


FIG. 96.—*Spirogyra majuscula*.[1] A cell of a thread gradually focussed into, showing therefore, besides the chlorophyll bands, the nucleus with its suspending threads (× 240).

[1] The synonymy of the *Spirogyra majuscula* of the text is rather obscure. This figure does not resemble *S. majuscula* of Kutz. = *S. orthospira*, of Näg. and Archer. The most common of the thick-threaded Spirogyras in Britain is *S. nitida*. The figure more resembles *S. orbicularis* of Hassall.—[ED.]

take a dark brown colour, and the irregularly serrate or jagged edges of the chlorophyll bands become very distinct. The central **nucleus** in this species is spindle-shaped, but by pressure upon the cell it is forced out of position, and shows a side view, and then presents the form of a disk; it has, therefore, in reality the form of a bi-convex lens. In its centre lies a large distinct **nucleolus**; seldom two or three such bodies are distributed symmetrically in the interior of the nucleus. In other nearly allied species the nucleus is thicker, and appears, in its natural position in the cell, to be rectangular, with rounded corners. The nucleus is surrounded by a very thin layer of protoplasm, from which delicate **protoplasmic threads** run out towards the peripheral protoplasm of the cell. By these threads the nucleus is suspended in the cell-sap which fills the cavity of the cell. The threads all arise from the thin rim of the nucleus, usually fork repeatedly in their course and join on to the inner side of the chlorophyll bands, and in all cases at the projecting parts which cover the pyrenoids. We can readily convince ourselves of this in most cases by slowly changing the focus. In individual cells the nucleus may have a position not central. This may indicate a late stage of cell-division, in which the new cell-wall shows clearly, with its thick cytoplasmic layer on each side, and the two daughter nuclei lying very near to it on either side.

Plasmolytic Studies in Spirogyra.—From the striking character and distribution of its cell-contents, Spirogyra offers a very favourable material for the study of **plasmolysis**, in which not merely the ordinary phenomena, but the remarkable capacity for resistance of the vacuolar protoplasmic membrane, is displayed. If a 10 per cent. solution of saltpetre, which is coloured with a little eosin, is permitted to act slowly upon this alga, in most cells a normal plasmolysis is seen to be induced. The protoplasm withdraws at first from the angles, then from the end surfaces, and ultimately from the side-walls, and contracts into a more or less spherical body. The eosin colour passes through the cell-wall up to the contracted protoplasm, but does not at first penetrate it. This is due to the resistance of the outer protoplasmic membrane, the ectoplasm. After from one to two hours, but perhaps sooner, the outer layers of the protoplasm begin to die, and to then accumulate the colouring matter. The wall of the vacuole, enclosing the now concentrated cell-sap, shows, however, still no change, and is impassable by the eosin; and the vacuoles are

therefore recognisable as colourless balls in the general pale red of the cell. Under the pressure of the dead outer plasmic mass the vacuole is often constricted, or the dead plasm may peel off, and in part lay the vacuolar membrane bare. This resisting inner layer may often remain living for days; de Vries has named it **tonoplast**, as it is by it that the pressure of the cell-sap is controlled. By a too rapid action of the saltpetre solution the outer plasm may be killed without contraction, only the wall of the vacuole remaining living; and this may withdraw from the dead plasmic masses and may contract itself into balls. Ordinary plasmolysis may also be effected with 5 per cent. or weaker solution of table salt. Protoplasmic threads not infrequently connect the contracted protoplasm with the end-walls of the cells.

NOTE ON THE USE OF ALCOHOL MATERIAL OF FUCUS (p. 241).

This cuts well if left in a mixture of 3 parts alcohol and 1 part glycerine for 24 hours.

If sections are placed in *pure* glycerine they swell slowly and assume a natural shape and appearance. They can then be mounted in glycerine-jelly.

This swelling in glycerine should be carried out upon the micro-slide, as the swollen sections cannot be readily moved.

They can be stained with alcoholic stains. Acetic gentian violet is said to be best (J. Chalon).

CHAPTER XX.

DIATOMACEÆ, PROTOCOCCUS, SCHIZOPHYCEÆ (FISSION ALGÆ).

Principal Materials Used.

Any large Diatom, *e.g.*, *Pinnularia* (*Navicula*) *viridis;* fresh.
Protococcus viridis, from damp bark of trees; fresh. Also *Hæmatococcus pluvialis*, from water-butt or roof-gutters.
Azolla caroliniana, for the Nostoc, *Anabæna Azollæ;* fresh. Or other *Nostoc* species.
Oscillaria species, from standing water or wet soil; fresh.
Gleocapsa species, from damp walls, rocks, or greenhouse pots or glass; fresh.

Principal Reagents Used.

Concentrated hydrochloric acid—Concentrated sulphuric acid—20 per cent. chromic acid—Concentrated chromic acid—Potassium permanganate—Calcined magnesia—Iodine—Chlorzinc iodine.

Structure of Diatoms.—The Diatomaceæ, or Bacillariaceæ, are unicellular organisms which form a somewhat isolated group in the organic world, showing their chief relationships, however, with Desmideæ, amongst Algæ. The most favourable object upon which to study the structure of the Diatomaceæ is, perhaps, *Pinnularia* (*Navicula*) *viridis*, a species very common in standing and flowing fresh water. It is distinguished amongst fresh-water forms by its comparatively large size, and allows in general an easy insight into its structural relations. Under the microscope, in which we must study them with the strongest objective at our command, they appear either in the form of an elongated ellipse, or as a rectangle with somewhat rounded angles. In the former case, we see them from the side of the **valve, test,** or **frustule** (Fig. 97, *A*), in the latter, of the **girdle,** or union of the valves (Fig. 97, *B*). We will call these the **valve-side** and **girdle-side** respectively. On the valve-side, the cell-wall appears marked with

narrow furrows, running from the edges towards, but without reaching, the middle (compare the figure). They are now usually considered to be chambers in the valve, open towards the interior. The central, smooth space, free from the furrows, shows at its middle and each end, a strongly-refractive thickening, which we distinguish as a **nodule.** The two end nodules are joined to the median nodule by a line, which bends out symmetrically close on either side of the median nodule, and ends in a slight enlargement. The end nodules are surrounded by the ends of the line in the form of a crescent, to effect which the line bends out at both ends laterally in the same direction as at the median nodule. In its course between the nodules, the line broadens a little. We assume that it is a cleft leading into the interior of the cell; it is the **raphe.** The furrows do not pass across the girdle-side (*B*); we see them only at the edges of the figure. By focussing for the optical section, and careful examination of the ends of the cell, we can demonstrate the remarkable fact that a median strip of the wall is double. From exhaustive investigation, it is determined that there is here an overlapping, boxwise, of separated parts of the wall. At the edges of the two elliptic wall-segments, which we saw in the view of the valve-side, portions of membrane adjoin, which end with free margins. The wall of this cell, therefore, consists of two halves, of which the one is inserted inside the other. The structure of this wall corresponds throughout to an elliptic box with a cover placed upon it. The side walls of the cover are just as high (deep) as those of the box, but they are not completely slipped the one into the other. If, in our cell, we pass from the optical section to the surface view, we can follow the thin edges of the two halves of the cell as delicate lines. The flat, furrowed surfaces of the cell-wall we distinguished as **valves,** their smooth free-ending side-walls **girdles;** whence the use of the terms in question to indicate

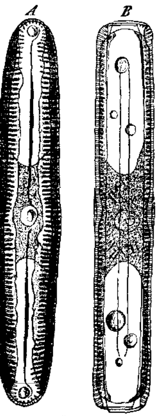


FIG. 97.—*Pinnularia viridis.* *A*, view of the valve-side. *B*, view of the girdle-side (× 540).

the two views. In *Pinnularia* it is easy to free the one half of the cell-wall from the other by pressure or by chemical reagents, and, moreover, here and there dead specimens are found in which this process has more or less completely taken place. With pressure the girdles easily break at some little distance from their edge, and along a line parallel with it. These lines, one near each edge, and therefore two in girdle-view, are often recognisable, and may be thin parts of the girdle. They do not extend to the ends of the cell.

Cell-contents.—The contents of the cell present a somewhat different appearance according to whether we have a valve- or girdle-view. In the former (Fig. 97, *A*), a median clear strip traverses the cell from end to end; the colourless **cytoplasm** of the cell is therefore visible. In the mid-length of the cell it appears collected into a bi-concave protoplasmic bridge. In this "bridge" lies the **nucleus**, not always readily visible without the use of reagents, and with a comparatively large **nucleolus**. Bounding both sides of this clear band, with a tolerably smooth or undulating outline, are the brown-coloured chromatophores, the **endochrome plates**. These lie, therefore, on the girdle-sides. In the protoplasmic "bridge" can be seen narrow rodlets, connected in pairs, the significance of which is unknown. Lastly, in the **cell-sap** lie usually, but not always, larger and smaller **oil-drops**. In the girdle-view the cell-body appears uniformly brown, because here the chromatophore covers the whole colourless lining protoplasmic layer. Only at the two extreme ends of the cell does the colourless protoplasm come to view. The chromatophore is uniformly dense and uniformly coloured, without visible differentiation. In girdle-view also the central collection of protoplasm appears to have the form of a bi-concave bridge.

If we now look over our former preparation of *Cladophora*, we are pretty certain to find diatoms clinging to this alga. They were fixed and stained at the same time with the alga, and we shall see the stained nucleus beautifully in each cell.

Amongst a large number of examples of *Pinnularia* we may here and there find one double. These are **sister-cells**, which have recently resulted from the **division of a mother-cell**. They cling to one another with their valve-sides, and, if the wall is fully developed, we can determine that the girdles of the two inner

valves are inserted within the two outer valves. After division of the contents of the mother-cell, these inner halves of the wall are developed for each individual. Each cell, therefore, has an older and a younger half of its wall, *i.e.*, one valve, the outer one, belonged to the mother-cell, and the other, inner valve, is peculiar to the present individual; and further consideration shows that the difference of age between the two valves may thus be very considerable.

Movements of Diatoms.—The *Pinnularia* cells are motile. They usually progress in the direction of their long axis, either uniformly, or by jerks, also turning off now and then laterally from their path. Only those diatoms which are provided with a raphe are capable of movement; to all appearance it is caused by a plasmic stream which moves in the raphe.

Siliceous Skeleton of Diatoms.—We now place a preparation of *Pinnularia* on a plate of mica, and heat it over a gas or spirit flame. We then lay the plate of mica, when cold, upon our object-slide, and observe the preparation dry, but under a cover-glass, with strong magnifying power. We can see that the *Pinnularia* remain as perfect skeletons. With short heating they become somewhat brown, from the carbonised organic substances; with longer continued heating they are colourless. Hydrochloric acid does not touch them; they consist of silica, and retain and show the finest peculiarities of the cell-wall, which must therefore have been silicified in a high degree. The furrows show in this preparation very clearly as dark striæ, and they are extremely good for studying other structural peculiarities of the wall. Especially beautifully visible in valve-view are the clefts, which run on both sides from the median nodules to the terminal nodules. Their broadening at mid-length is manifest. In the girdle-view the edges of the two halves of the cell-wall show clearly; moreover, on the overlapping parts are seen two lines parallel with one another and with the edges of the valves, which do not extend to the ends of the cell.—Flint-skeletons quite as beautiful as these are also obtained if we first allow a drop of concentrated sulphuric acid to act upon our diatoms, and after some time add 20 per cent., and then gradually concentrated chromic acid, and finally remove these reagents with water. Diatom valves which are poor in silica will neither bear heating red-hot, nor this last method of procedure; they must instead be laid for from four to seven days in hydrochloric acid, to which a

little chlorate of potash has been added; and it is still advisable after this to lay the material for two days in ammonia, and afterwards transfer to nitric acid.

A very simple method of preparation, by which the cell-contents of the diatoms are dissolved, and good valves as a rule obtained, is treatment by potassium permanganate. Crystals of this salt are added to the fresh diatomaceous material, in the proportions of about 1 part of the salt to 10 parts water, and the mixture exposed in an evaporating dish of about 100 c.c. capacity either on the hot plate of an oven or in the sun, and from time to time stirred. The vessel is then half-filled with water and about 50 cg. calcined magnesia is stirred in. After from two to three hours about 1 cc. hydrochloric acid is poured in each ten minutes, the operation being complete when the contents of the vessel are decolorised.

The richest diatom gatherings can be made in spring and autumn. Rusty-looking and yellow-brown slimy coatings upon earth and stones, in relatively clear standing and flowing waters, in mill-sluices, and on mill-wheels, indicate diatoms. They cling to water-plants, and a compact turf of moss upon damp rocks will conceal them in great numbers.

On account of the often exceedingly delicate and regular structural relations of their cell-walls, diatoms are much used as test objects, in guaging the quality of the more powerful microscopic objectives. Especially used are the valves of *Pleurosigma angulatum*, which, with sufficiently strong magnification, show regularly-arranged hexagons.

Protococcus viridis.—In order to become acquainted with one of the simplest possible forms of the unicellular green algæ we will examine a *Protococcus*. To this group belong in the main all the green incrustations which are found on the stems of trees, damp boards (*e.g.*, wood palings, etc.), walls, and other similar places; but we must note that it is quite uncertain whether our *Protococcus* is an independent species, or is to be considered as a stage in the development of another alga. The form (Fig. 98) which we take from an old tree trunk comes under the name *Protococcus viridis* (*Pleurococcus vulgaris*). We examine it with a strong magnifying power, and find it composed of globular cells, isolated or united into small groups (Fig. 98, *A* to *F*). The contents of the cells are bright green, but the whole protoplasm is not uniformly coloured, for, as sufficiently strong magnification

shows, a number of **chromatophores** are present, which, in lateral contact, occupy the exterior of the cell-contents. Where their contact is not complete, the colourless embedding protoplasm comes into view. More or less in the middle of the cell, but not usually visible without the help of reagents, lies the **nucleus**, with its **nucleolus**. The cells have a thin wall, which stains violet with chlorzinc iodine. Numerous cells are usually in course of bipartition by means of a partition wall, which cuts the spherical cell into halves (Fig. 98, *D*). The divisions of adjoining cells take place in planes either parallel or cutting one another at right angles. The daughter-cells, becoming rounded off, soon pass out of union with one another (*C*, *F*); they may remain for some time clinging together, or else become at once separated. If the cells are treated with potassium-iodide-iodine, the nuclei show up clearly (our figures were sketched from such preparations). In each nucleus the nucleolus is then clearly visible. In the cells which have just arisen by division, the nuclei lie against the young partition wall (*D*). The iodine solution shows small starch grains in the chromatophores, but no pyrenoids.

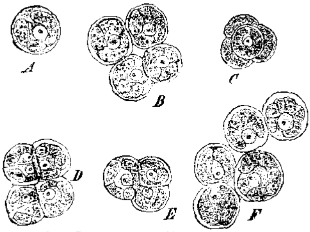


FIG. 98.—*Protococcus viridis*, after treatment with potassium-iodide-iodine. In *D* the cells on the left have just divided (× 540).

We have said that this alga is by some considered to be only a stage in the life history of some higher form, probably one of the Volvocineæ. If some of this material is placed in water and exposed to the light, some of the cells will enter upon another phase of their existence, becoming motile by means of cilia. More suitable for microscopical study, however, is another form of *Protococcus*, commonly known as *Protococcus* (or *Hæmatococcus*) *pluvialis*. This *Protococcus* is found very widely distributed in rain-water, in the mud at the bottom of open water-butts, in roof-gutters, etc.

Structure of Nostoc.—The irregular, folded or lobed, olive-green masses of jelly, sometimes found on damp paths, appertain to one of the Fission Algæ, or Cyanophyceæ (Schizophyceæ) known as *Nostoc ciniflonum*, Tournf. (*N. commune*, Vauch). Other species,

forming smaller, often very small, jelly-masses are frequent in damp places or in water, or living endophytically, in association (possibly symbiotic) with other plants, such as the roots of Cycads, in hollows of certain Liverworts, in the small greenhouse floating aquatic plant *Azolla caroliniana* and the duck-weeds (*Lemna*), and in true symbiosis in the case of some Lichens such as Collema. If a small portion of such a mass of jelly be placed under the microscope we find it traversed hither and thither by twisted and coiled necklace-like threads of small cells (see Fig. 99, *Anabæna Azollæ*, endophytic in *Azolla caroliniana*). The globular or barrel-shaped cells of which the thread is composed, enclose very minute verdigris-green chromatophores, so closely placed together in the neighbourhood of the cell-wall that the living layer of cytoplasm appears to be uniformly coloured; and also show small rounded bodies which can be distinguished as cyanophycin grains, oil drops, glycogen and vacuoles. The interior of the cell shows the so-called **central body**, which by many is considered to be of the nature of a nucleus. It differs from the nucleus of the higher plants in the want of clear delimitation from the surrounding cytoplasm, and the absence of nucleolular bodies; when in division, however, it shows structures which are suggestive of the "mitotic figures" of typical nuclei (see Chapter XXXII.).[1]

Many cells will be found in division. These are longer, and show a shallow median constriction (Fig. 99, *a*, *b*). To this annular constriction succeeds the formation, progressively from without inwards, of a division wall (*c*). The cell-walls are very delicate. A colourless homogeneous jelly arises from the progressive swelling of the outer layers, and in this jelly the threads lie embedded; it is more or less completely wanting, however, in many endophytic forms. Here and there in the threads will be seen single larger, globular cells (*h*), which have a thicker cell-wall, appear yellowish or brownish in colour, and have very reduced homogeneous contents. These are the so-called **heterocysts**, which are incapable of division and of unknown function. At the points of attachment to the adjoining cells the heterocyst shows small projecting bosses. Chitin has been shown to be present in the membrane of the Cyanophyceæ, excepting in the heterocysts.

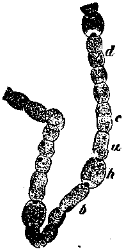


FIG. 99.—*Anabæna Azollæ*. *a* to *d*, successive stages in the division of vegetative cells; *h*, a limited cell, or heterocyst (× 540).

[1] See Wager, *Proc. R.S.*, 1903, p. 401.

Oscillaria.—In examining any terrestrial form of *Vaucheria*, particularly that collected from flower-pots, we meet with *Oscillaria*, likewise belonging to the Schizophyta (splitting plants), in closest affinity to the Nostocaceæ. They are found, moreover, almost everywhere in standing water, on muddy ground, etc. Their presence is often betrayed by an unpleasant muddy smell. *Cultivated in vessels, they creep in part to the walls of this, over the* surface of the water. They are nearly straight, or may be even coiled threads, coloured from blue-green, verdigris-green, olive-green to brown; can, however, be colourless, and are often distinguished by active motility. The threads are free, or enclosed in a gelatinous sheath. They can be inserted individually, or in numbers, in such a sheath. The sheaths arise from the gelatinous outer layers of the membrane of the threads; where these layers become diffluent, the sheaths are wanting. The threads are divided by cross partition walls into short disk-like cells. The partition walls in some species can be seen very easily, in others with great difficulty. The cell-contents are in general agreement with those of *Nostoc*.

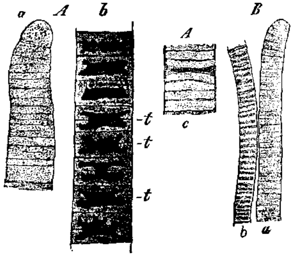


FIG. 100.—*A*, *Oscillaria princeps*; *B*, *Oscillaria Froelichii*; *a*, ends of the threads; *b*, piece from the middle of the thread; *t*, cells in division; in *B*, *b*, the granules especially collected against the partition walls; in *A*, *c* is a dead cell between two living ones. The segment *A*, *b is after fixing in chromic acid*, and staining with methylblue-eosin; the other segments are from living specimens (*A* × 1080, *B* × 540).

It matters nothing what species is used for examination, but the thicker forms, with more distinct partition walls, have the preference (see Fig. 100). With suitable treatment, the **central body** can be quickly made recognisable. First wash the threads in water to free them from impurities; then transfer them to 1 per cent. chromic acid, in which they should be left for 5 minutes. Again wash in water, and leave for about 3 minutes in a concentrated watery solution of 4 parts methyl blue and 1 part eosin. After rinsing in water the threads can be examined in water. We can also allow the threads to dry upon a micro-slide, and after they are completely air-dry can put a drop of Canada balsam upon them, and thus

make thoroughly useful permanent preparations. An *Oscillaria* thread, thus fixed and stained, is shown in Fig. 100, *A*, *b*. The central bodies are stained blue, the cytoplasm is rose-red. Individual cells (*t*, *t*) are in course of division; they show the central body dividing, and the cell-wall intruding between the halves.

Movements of Oscillaria.—The phenomena of movement, as we must have noticed from the very beginning of our observation of the Oscillariæ, are very interesting; and with a sufficiently strong power we shall be able to study the phenomena best in the thicker forms, with bent end and distinct granules. We then determine that with the movement of the thread is combined a slow rotation on its axis. Simultaneously the thread shows irregular flexions, or nutations, which are the expression of differences in the rate of growth on its different sides. These flexions usually take place slowly; but violent movements can be induced when the flexion is stopped by some obstacle, and, by overcoming this, the tension is suddenly relieved. The Oscillaria-threads move now forwards, now backwards. The movements can only take place when the threads are in contact with some other object. The straight threads move like those which are bent; in these latter the phenomenon is, however, especially striking, and at once visible, while in the straight threads it is necessary to fix the attention upon individual granules, in order to demonstrate a rotation of the thread on its axis.

Structure of Glœocapsa.—To the same class of organisms as the Nostocaceæ and the Oscillariæ belong the still more simply constructed Chroöcoccaceæ, which we will study upon one of the widely-distributed species of *Glœocapsa*. We choose *G. polydermatica*[1] (Fig. 101), growing upon damp walls or rocks, recognisable as a dirty-green to olive-green gelatinous layer. Under the microscope we find a gelatinous envelope divided by delicate walls, showing lamination; and within it uniformly-coloured cells, more or less clearly granular, and with central body. By these peculiarities of their cell-body the Chroöcoccaceæ are distinguished from Protococcaceæ and especially Palmellaceæ, which in many forms very strongly resemble them, but which have a nucleus, and chromatophores, separated from the rest of the protoplasmic body.

[1] More readily obtainable, and very similar, is *G. caldariorum*, a species growing commonly on the walls, flower-pots and glass, etc., in conservatories and greenhouses.—[Ed.]

In *Glœocapsa polydermatica* the cells which have arisen from quite recent division are quite globular (Fig. 101, *C*). They then begin to grow in length and become elliptic. They then show a slight hour-glass-like constriction (*A*) in mid-length, after which a delicate partition wall becomes visible at this place, simultaneously with the formation of which the young cells form a delicate wall over the rest of their surface. The daughter-cells now round off, and on both arises a relatively thick gelatinous layer, by which they become separated from one another. Thin cell-walls and thicker gelatinous layers thus alternate with one another, and a considerable number of generations are therefore combined by the gelatinous envelopes into a single cell-family or colony. By rupture of the outer envelopes the families fall apart. With careful examination we can in general determine that the number of the thin cell-walls, which their stronger refractiveness has made visible, does not correspond with the number of cell-generations enclosed within. Usually, it is true, the formation of one such cell-wall follows the division of the cell-contents; nevertheless, two or more such cell-walls can be intercalated in the gelatinous wall between two stages of division. An isolated cell is occasionally found, and then is usually surrounded by a considerable number of gelatinous envelopes (Fig. 101, *A*). In such cases the cell-division is discontinued, not the thickening of the wall.

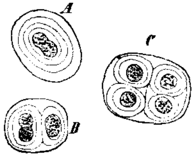


FIG. 101.—*Glœocapsa polydermatica*. In *A*, at the commencement of division; in *B*, to the left, shortly after division (× 540).

NOTE ON THE CULTURE OF ALGÆ.

In a short paper in *Annals of Botany*, v. 13, p. 568 (1899), Marshall Ward gives various methods for cultivating and isolating such simple Algæ as *Oscillaria*, *Palmella*, some Protococcoideæ. The description does not lend itself to further condensation, and the paper should be referred to.

NOTE ON *PROTOCOCCUS VIRIDIS* (*PLEUROCOCCUS VULGARIS*), (p. 260).

The incrustation formed by this plant is usually on the windward side. According to Chodat (*Annals of Botany*, v. xi., p. 115), the chloroplast is a single deeply lobed parietal plate, which contains a prominent pyrenoid; and this view is confirmed by G. S. West (*Brit. Fresh Water Algæ*, 1904, p. 203). In moist places, short filaments of cells are sometimes produced which exhibit a simple type of branching; they frequently radiate from a few central cells of an angular or more or less parenchymatous form. This condition is readily produced in cultures, and can be described as the *protoderma* stage.

CHAPTER XXI.

SCHIZOMYCETES[1] (BACTERIA)—SACCHAROMYCES (YEAST).

PRINCIPAL MATERIALS USED.

Spontaneous cultures of various Bacteria in infusion of lettuce (or other) leaves, and of peas, and on discs of boiled carrot, turnip and potato.
Fur of teeth.
Water with decaying Confervæ; or filtered infusion of pea-flour, decomposing; for *Spirochæte.*
Hay-infusion; for *Bacillus subtilis.*
Decaying peas or beans for *Bacterium Termo.*
Brewer's Yeast (barm).
Many special materials and appliances may be used, for which see text.

PRINCIPAL REAGENTS USED.

Indian ink—Iodine—9 per cent. potash—A stain, such as methyl violet, gentian violet, fuchsin, or methylene blue—Ehrlich's aniline-water-fuchsin and methylene blue—Many special reagents and stains are used, and will be found described in the text.

LET us now turn our attention to examples of the group of smallest known organisms, the Bacteria, in order to obtain some general information as to the forms they may assume. The great importance which has in recent times been attached to these organisms, as playing an active part in many important industries, but especially as a means of transmission of infectious diseases, has resulted in an enormous expansion of their study. Bacteriology has become a special branch of botanical and medical science, and is treated in numerous comprehensive treatises.[2]

[1] Segmenting, splitting or fission-fungi. As a convenient term for an individual, not implying any special kind, I use the word "bacteriad".—[ED.]

[2] An exceptionally valuable example of the scientific method of treating a bacteriological problem is found in H. Marshall Ward, "The Ginger-beer Plant, and the organisms composing it: a contribution to the study of Fermentation-Yeasts and Bacteria"; *Phil. Trans.*, 1892 B, pp. 125-197, and Plates 11-16.—[Ed.]

These concern themselves chiefly with the microscopical distinction of bacteria by means of stains, with pure cultures of them, and with their pathological action. Those who wish to undertake special bacteriological studies should turn to these works; in this chapter our intention is merely to introduce the beginner to the study of bacteria, to make known to him the botanical side of the problem, to teach him some of the most important methods of staining, and to give him an insight into the technics of pure culture.

General Notions.—We will first of all fix our attention upon certain universally-distributed bacterial forms, in order to obtain some generalised information about the group. We shall not endeavour at first to study any particular species; we will rather leave to accident what form may be at our disposal.

We boil some green leaves, say of the lettuce, in a Florence flask, and leave it standing open at a comparatively high temperature. Into another flask, with a little water, we place some peas, killed by steeping in boiling water. At the same time we prepare disks of boiled carrot, turnip and potato, on watch-glasses or object-slides, and place them about in warm, moderately moist, places, some free, others covered with glass bell-jars. Upon the infusion of leaves, after two days (or longer if the situation is cooler), a skin may have been formed, which we will call the **pellicle.** On the different vegetable disks we see small whitish, rarely coloured, spots of gelatinous substance appear, enlarge, fuse together; on the potato disks these structures mostly take the form of a white, later on grey, and ultimately brown, wrinkled skin.—We bring a trace of any one of these spots of jelly into a drop of distilled water on an object-slide. This is best effected by means of a bit of platinum wire, fused into a glass-rod for a handle, and the free end of which is curved round into a small loop. Before use this must be heated red hot in a spirit flame, and used immediately when cold, in order to prevent the inclusion of other bacteria which may be about. Cover the drop with a very thin cover-glass, and examine it with the strongest possible magnification; we find an enormous number of exceedingly minute bodies, appearing almost dot-like, embedded in the jelly. These bodies show a necklace-like arrangement; but we also find them singly, or in pairs, or united in large numbers into a string. Embedded in the jelly, then, we have the **Coccus**-form of some kind of bacteriad. If

we wish to define the outer limits of the jelly, which, in its refractive relations, differs only little from water, we can readily do so with the aid of Indian ink. The ink must be of good quality, and should be ground down very carefully in water. A drop of this ink can be placed upon the object-slide, the gelatinous mass which is to be investigated placed upon a cover-glass, and the cover-glass then laid upon the drop. In this way the particles of ink are prevented from passing between the jelly and the cover-glass. The limits of the jelly are now sharply defined, on account of the surrounding fluid being filled with fine particles of ink—which exert no injurious influence on the object. Such masses of bacteria, embedded in jelly, are distinguished as **zooglœa**, or the **zooglœa stage** of the bacteriad. The jelly arises from the swollen membranes of the bacteria, and in a number of forms carbohydrates have been determined in it.—It is quite possible that the jelly taken into observation does not contain these round "cocci," but shorter or longer rodlets (compare Figs. 102 and 104 *A*). The longer rodlets can be identified as composed of shorter segments, which stand out very clearly if we add iodine solution to the preparation. The segments now appear much shorter than when in the fresh state, and partition walls are shown which formerly were invisible. Such figures enable us to form an opinion as to their mode of multiplication; this takes place by successive bipartition or fission, and the method has given to bacteria the name of fission-fungi or Schizomycetes. The divisions follow one another in the same direction, approximately at like distances, and at right angles to the long axis of the organism. Only in a few cases, *e.g.*, the *Sarcina* of the stomach, do divisions take place at right angles with one another.

The **contents** of the bacterial cells show a *protoplasmic lining* layer and a vacuole, which in the elongated forms is broken up by protoplasmic partitions or diaphragms. In the lining layer are one or many small bodies which by their relations with stains are recognised as *chromatin grains*. Some consider these to be of the nature of a distributed nucleus. The presence of a single central nucleus has been stated; but undoubted nuclear division (Karyokinesis) has not been seen, so that the presence of nuclei cannot yet (1906) be asserted.

The **protoplasm** of the living bacterial cell is in general colourless; only in a very few cases does it show a green colour from chlorophyll (*Bacillus viridis*), or bright red (*Beggiatoa roseo-persicina*), etc. With macroscopic observation, on the other hand,

the bacterial masses often show definite colour, grey, yellowish and even stronger colours. These colours are usually, if not exclusively, confined to the membrane, and often serve for the macroscopic distinction of the species. It is quite possible that upon our vegetable disks such a strongly-coloured form may appear; for example *Micrococcus prodigiosus*—looking like drops of blood.

Some bacteria are distinguished by the formation of an amyloid in their body, either in the stages preceding spore-formation (as *Bacillus Amylobacter*), or independently of spore-formation (as *Bacterium Pastorianum*, and, occasionally, *Bacillus buccalis*); and after addition of iodine solution either the entire mass, or to the exclusion of definite cross-zones, stains blue to violet.

Certain bacteria, known collectively as "sulphur bacteria," contain sulphur granules of viscous consistence in their interior; others show iron-oxide deposits in their envelope, and are distinguished as "iron bacteria". The iron-oxide deposits give to the iron bacteria a brownish colour.

In the pellicle which comes upon the surface of the leaf-infusion we have also a form of Zooglœa (*cf.* Fig. 104 *A*); in it the cell-rows are held together by jelly. This pellicle is traversed by delicate, undulating, more or less parallel threads, formed of cocci or, usually, of rodlets. Here also the segmentation of the cocci or rodlets shows up specially clearly after the addition of iodine solution. In a preparation taken from this culture we often see the **swarming** stage of development; but such can be obtained with certainty in water in which peas have been soaked for one or two days. We see then the bacteria in question in dancing movement, hurrying about, now forwards, now backwards, in various directions. Extremely fine cilia have been distinguished upon swarming bacteria, and it is supposed that the movement is brought about by their means (see Fig. 105).

In exhausted nutrient substrata changes of form and contents of the bacteria not infrequently come. These are considered to be pathological or diseased conditions, prior to death, and the forms showing then are distinguished as "**involution forms**" (Nägeli).

If we now examine the pellicle of a leaf-infusion, such as above, which has already stood for several days, we shall perhaps find the rodlets or threads in **spore-formation** (Fig. 104 *C*). The contents of the cells have collected at individual points, not more than

one in each cell, and form rounded or elliptic strongly refractive bodies, which appear as dark granules, and represent **resting-spores.** These persist, while the emptied membranes of the rodlets ultimately are decomposed. In material from other cultures we shall quite as commonly find rodlets which form the resting-spore at one of their ends, and hence take the appearance of a pin or tadpole. Many bacteria form these end-spores quite regularly, while others again are characterised by swollen spindle-formed spores in their centre.

Those bacteria which form spores in the interior of their cells are collected together as **endosporous bacteria**; while in **arthrosporous bacteria** entire cells take on the character of spores. These latter remain, while the rest of the cells perish. This conduct holds good in general for those bacteria which at the time of spore-formation consist of spherical members; while the spore-forming rods and threads are especially endosporous. Endosporous spore-formation is, indeed, essentially confined to the rodlet-bacteria; only in individual cases can it be also observed in the spirally-twisted **Spirillum.** In very numerous bacterial forms spore-formation cannot in general be found. The arthrosporously-developed spores must not be looked upon as quite equivalent to those produced endosporously, but rather as only a resting state of the bacteria in question.

It is now certain that bacteria have only a very simple cycle of development. The **polymorphism** (or **pleomorphism**) which was formerly asserted to exist does not, as more recent researches show, occur even in the most highly-differentiated sulphur and iron bacteria—those in which distinction between apex and base is shown.

General Reactions.—In fluid substances which one examines for bacteria, granulations of various kinds may render observation more difficult, and give rise to false impressions. We endeavour, therefore, in the first place, to obtain as much information as possible from the fresh objects, and then, before having recourse to stains, call in the aid of certain reagents. Though we can use these direct, upon the fresh moist preparations, it is usually preferable to first dry them. To effect this, spread a little of the fluid uniformly upon a cover-glass, in the thinnest possible layer, by means of the sterilised looped platinum wire already referred to; or upon this cover-glass thus prepared another can be laid, so that the material is spread out between them, and the two separated

by fingers or forceps, each being covered with a very thin film of the material. The cover-glasses should remain in a chamber free of dust until they are completely air-dry.

Bacteria show themselves to be very resistant to water, alcohol, ether, dilute mineral acids, acetic acid and weak alkalies, and we can proceed to test them with these reagents. We make use of 50 per cent. acetic acid, or 12 per cent. sulphuric acid, or, better still, 3 per cent. potash. In this last the preparation becomes at once as transparent as necessary, the bacteria usually standing out quite sharply; and, by swelling, their bulk is so far enlarged that a less strong magnification is made useable. Since large masses of oil, should they be in the preparation, injure observation very greatly, we should take care to remove them. This can be effected either by warming the dry preparation, covered with a drop of potash over a spirit flame, until bubbles begin to be formed, by which means the fat is saponified; or the dry preparation can be treated for a few minutes in a watch-glass with chloroform, and then with absolute alcohol, and after the evaporation of this latter the potash applied.

An exception to this capacity for resistance possessed by bacteria is afforded, amongst others, by *Spirillum* (*Spirochæte*) *Obermeieri*, the spirillum of intermittent fever, and some other *Spirillum*, which are destroyed by these reagents. We can, however, in general decide that regularly-formed bodies, which resist the action of alcohol and ether, the acetic acid and potash tests (even with warming, as above), can be considered as bacteria; although in certain cases deception by specially resistant and regular granulations is not entirely excluded from the range of possibilities.

Staining Methods.—Their staining properties are also of value in the determination of bacteria. It is true that other minute bacteria-like bodies can take up stains, and some bacteria will not stain at once, so that here also in some circumstances caution is necessary. Specially used are the so-called basic aniline colours, and above all methyl violet, gentian violet, methylene blue, fuchsin, Bismarck brown and Vesuvin, are used for the staining of bacteria. The bacteria not only take up these stains eagerly, but retain them very energetically, far more energetically than they are at the same time retained by the neighbouring tissue elements in the preparation, if the bacteria are stained *in situ*. These stains are used in saturated watery solutions, which must

be either freshly prepared or at least freshly filtered, or in dilute alcoholic solutions. In order to prepare these latter we make saturated alcoholic solutions of the stains, preferably in drop-bottles, *and add them drop by drop to a considerable bulk of* distilled water, which should be at least tenfold that of the alcoholic solution. Watery methyl violet, gentian violet, or fuchsin solution must always be freshly prepared, while dilute methylene-blue solution keeps. Bismarck brown or Vesuvin changes in alcohol, and must therefore be kept in a watery solution, but must always be filtered before use; but the other stains are to be preferred to them. The bacteria in a fluid medium are spread out on a cover-glass, in the thinnest possible layer, and allowed to dry, exactly as is described above, at room temperature and protected from dust. If the fluid contains albuminous bodies or mucilage, these, after complete drying of the preparation, must be fixed, which can be effected either by laying the cover-glass for several days in absolute alcohol, or at once by high temperature. This latter method, which is very rapid and convenient, can be carried out by holding the dried cover-glass by means of forceps (best of all the flat-ended, bent forceps with crossed legs, so as to open with pressure, and known as "cover-glass forceps"), and passing it about three times moderately quickly through the flame *of a Bunsen burner, or a strong spirit-lamp flame, the bacteria-*covered side being held upwards. Too long action of the flame injures the staining properties of the bacteria, so that it is desirable not to too greatly prolong it. The preparation is stained by spreading upon the cover-glass, prepared in this kind of way (and which must in all cases be air-dry), some drops of the stain, and allowing it to act for a few seconds, though in some cases up to five minutes is necessary. With bacteria which take the stain badly, warming the staining fluid is an assistance. For this we hold the cover-glass in the flame with the staining solution upon it, till the fluid begins to steam. After staining, the cover-glass is rinsed in distilled water, its clear side dried with a linen rag, and the preparation may then be at once examined in water; or, after rinsing, and drying the clear side, the preparation may be dried at room temperature, cleared by means of a drop of turpentine, xylol, oil of cedar, or oil of bergamot placed upon it, and examined in this. If the bacteria are found to be overstained, a part of the colour can be removed if absolute alcohol is allowed to act upon it for a sufficient time. The same result can be attained

if oil of cloves is used for clearing, for this extracts the colouring matter, more or less completely, according to the time during which action is permitted. For the same purpose it has also been recommended to immerse the preparation (after rinsing in water) for one second in 0·5 per cent. acetic acid. Overstained preparations, from which a portion of the colour has been removed, often give the most beautiful figures. If it is to be kept, the clearing fluid should be removed by blotting-paper, and the preparation mounted preferably in Canada balsam. The Canada balsam should, however, be dissolved in xylol or in turpentine, not in chloroform, since this latter extracts these basic aniline colours; and for the same reason the Canada balsam must not be used warm. For permanent preparations a little overstaining is no disadvantage, since in Canada balsam the depth of the colour in time diminishes. If the preparation should ever be used with a homogeneous immersion lens, we must take care that the Canada balsam does not extend from under the edge of the cover-glass, since it is soluble in the immersion oil, and the entire cover-glass may thus be fouled. To prevent this the edge of the cover-glass can, after the Canada balsam is set, be covered with a narrow band of gold-size, which is insoluble in the immersion oil. This is done with a fine camel-hair brush, care being taken that the gold-size does not extend further than necessary over the cover-glass. Preparations stained in Bismarck brown or Vesuvin retain their colour also in glycerine, and can therefore be preserved in it. The closing of the edge of the cover-glass is then effected by means of Canada balsam dissolved in chloroform, and after some days or weeks, as convenient, the balsam can be covered with the band of gold-size as above. These latter stained preparations can also be embedded in glycerine-jelly, and then need no further closing.

If *material in spore-formation* has appeared in our culture, we may perhaps have noted in our attempts at staining that the spores remain unstained. This arises from our manipulations thus far not having led to the death of the spores, so that they do not absorb the stain. But if we pass the air-dry cover-glass preparation sufficiently often through the flame, say eight to ten times, the spores afterwards stain; but at the same time the other parts of the spore-forming bacteria, as well as those not fructifying, and also the cytoplasm and nuclei of any tissue elements in the preparation, lose more or less completely their capacity for staining. The same effect is produced if we allow the air-dry cover-

glass preparation to remain for a quarter to half an hour in a dry chamber at 180° to 200° C. In order to obtain simultaneously beautiful spore-staining, and also staining of the rest of the body of the bacteria, we can best use Ehrlich's aniline-water fuchsin method. This reagent is prepared by first shaking up purified aniline oil in excess with distilled water for about one minute. We can use about 5 c.c. aniline oil in 100 c.c. water. Let this stand for about five minutes, and then filter through a filter which has been previously damped with distilled water. The filtrate must be as clear as water, and it is recommended that it should always be freshly prepared. We then add to it an alcoholic solution of fuchsin until the fluid begins to become distinctly opalescent. The dried cover-glass preparation, after passing the suggested three times through the flame, is allowed to float for an hour upon this aniline-water-fuchsin solution, made hot. The spores and the protoplasm of the bacteria are then uniformly stained. It is recommended, however, to now pick out the spores from the cytoplasm by means of double staining; for which purpose, after use of the aniline-water-fuchsin, as above, a watery or dilute alcoholic solution of methylene blue is specially suited. This double staining is most certain if the superfluous aniline-water-fuchsin is removed by laying the preparation for a few seconds or minutes in absolute alcohol, or even in alcohol feebly acidulated with hydrochloric acid ($\frac{1}{2}$ per cent.), and the methylene blue then used. In many cases instead of alcohol or acidulated alcohol dilute mineral acids must be used in order to obtain quite sharp figures. A successful double-staining with aniline-water-fuchsin and methlylene blue shows the spores stained red, the rest of the bacterial body more or less dark blue. Instead of the aniline-water method, quite the same results follow the use of Ziehl's carbolic-fuchsin solution for spore staining. The solution is prepared by dissolving 1 part fuchsin in 100 parts 5 per cent. carbolic acid and 10 parts alcohol. Both solutions, Ehrlich's and Ziehl's, but especially the former, enjoy a very wide use for bacterial staining in general. Ehrlich's solution can also be prepared with gentian violet or with methyl violet, instead of with fuchsin. It only keeps for at the most a few days, and should in general not be used till twenty-four hours after preparation; Ziehl's solution on the other hand keeps. The staining capacity of Ehrlich's solution is heightened if to 100 c.c. of the saturated aniline water 1 c.c. of a 1 per cent. solution of caustic soda is

added, and afterwards, instead of an alcoholic solution of fuchsin, 4 to 5 grams of the solid dye, whether fuchsin, methyl violet, or methylene blue, is added, and the solution thoroughly shaken.

Staining in the Tissues.—Only in the most exceptional cases can it be recommended to examine sections from fresh tissues for bacteria. It is much more advantageous, and usually indeed necessary, to previously harden such tissues. For this hardening absolute alcohol is best, since the use of other hardening media makes the success of the subsequent staining less certain. Pieces of tissue to be hardened in alcohol should not be larger than a hazel-nut, and should lie for at least three days in a relatively large quantity of absolute alcohol or strongest methylated spirits. Not all bacteria can be equally readily stained. Staining, however, almost invariably results from the use of a strong alkaline solution of methylene blue, which may be considered the best all-round stain for bacteria. This solution is prepared if 30 c.c. concentrated alcoholic solution of methylene blue is added to 100 c.c. potash solution of strength 1 : 1000 ; it does not keep long. Staining is completed in a few minutes, and the sections are then washed in 0·5 per cent. acetic acid, dehydrated in alcohol, cleared in oil of cedar, and preserved in Canada balsam.

If it is desired to have only certain bacteria in the section stained, the rest, as well as the tissue, can be decolorised. In general the bacteria resist decolorising better than the tissue elements, but show, on the other hand, varying degrees of tenacity in colour-retention. For such "isolation," that is, the staining of particular bacteria, Gram's method is most commonly used. This is specially noteworthy for the fact that it brings about the decolorising of the nuclei in the cell-tissues without altering the colour of most bacteria. The sections are first stained with aniline-water-methyl-violet, or aniline-water-fuchsin, freshly prepared exactly as described above, *i.e.*, 5 c.c. pure aniline oil added to about 100 c.c. distilled water, thoroughly shaken, and filtered through a previously-damped filter. To this clear aniline water 11 c.c. of a concentrated alcoholic solution of methyl violet (better than the gentian violet which was at first recommended) are added, it is again filtered through a damped filter, and finally 10 c.c. absolute alcohol added. This solution keeps for about a fortnight. Sections stained with this fluid are transferred either direct, or after slight rinsing in alcohol, to an iodine solution which contains 2 parts potassium iodide and 1 part iodine to 300

parts distilled water. There they should remain from one to three minutes. As a result of the precipitation from the iodine solution the sections are dark purple. After transfer to alcohol the sections are decolorised; then they are placed in oil of cloves, and then in Canada balsam.—Somewhat of a modification of Gram's method, known as the Gram-Günther method, is now specially used. For decolorising, not only alcohol but also 3 per cent. hydrochloric acid alcohol is used. The sections, treated with iodine, are placed for half a minute in alcohol, about ten seconds *in 3 per cent.* hydrochloric acid alcohol, for several minutes again in fresh alcohol, and repeatedly in further quantities of clean alcohol till the maximum of decolorisation; ultimately, when no more colour comes out of the section, place it in xylol, and thence in Canada balsam dissolved in xylol. Certain bacteria, as well as the cell-nucleus will be deprived of their colour by this process. The staining relations in such a case can therefore serve for the distinguishing of bacteria by a kind of "differential diagnosis". Gram's method has also been found of use for cover-glass preparations.

In searching for bacteria in tissues, after staining, Abbe's substage condenser (or illuminating apparatus) can be used with great advantage, and in a quite special fashion. After focussing the preparation, the iris diaphragm is opened quite wide, so that the object is flooded by a cone of illumination of the full aperture of the objective. All unstained structures now disappear more or less completely, while the stained light-absorbing bodies remain visible. We may call this "isolation of the coloured image". Approximately similar effects are obtained with other substage condensers.

After these generalised considerations we will endeavour to study more closely the morphological characteristics of certain definite bacteria which are not difficult to procure.

Bacteria in the "fur" of Teeth.—We draw first upon a source which provides for us simultaneously pretty well all the characteristic bacterial forms—the white "fur" of the teeth. Numerous species of bacteria dwell therein, and we can reckon almost with certainty to obtain therefrom spherical bacteria, rodlets, threads and spirals (Fig. 102). If a small quantity of the "fur" is diffused in a drop of water, and examined with the highest possible power, we first see thicker threads in parallel bundles, and thinner ones

interwoven with them. After addition of iodine solution the thicker threads are coloured bluish-violet, the thinner ones yellow. The threads coloured blue violet (by reason of their amyloid contents) are distinguished as *Bacillus buccalis*, the others as *Leptothrix buccalis*. The iodine solution enables us to see clearly a segmentation of these threads into shorter joints. The chains of four to ten cells in a sheath, which we observe now and again, coloured blue violet with the iodine, has been named *Jodococcus vulgaris*. Then comes *Spirochæte dentium*, in long actively motile spirals (*c*); and lastly *Spirillum sputigenum* likewise actively motile, in "comma"-shaped rodlets often connected in pairs (*d*). Besides these, are incidentally found in the mouth a considerable number of other bacteria, in part as yet insufficiently studied. These bacteria live saprophytically upon the mucous membrane or the fur of the teeth, or on fragments of food; and some, at least, are concerned in promoting the decay of teeth, in that they penetrate into the calcified tissues of the teeth and destroy the softened bone. If a preparation be treated with 10 per cent. solution of common salt the bacteria are plasmolysed. This is best seen in the thick threads of *Bacillus buccalis*. The contents of individual cells of the rods are seen aggregated into one or more refractive masses, having quite the aspect of spores. At the same time the wall becomes recognisable. If the preparation has been in water for a little while a weaker solution, *e.g.*, 5 per cent., will induce plasmolysis.

FIG. 102.—Bacteria from the "fur" of teeth. *a*, *Bacillus buccalis* and *Leptothrix buccalis*, at *a** after treatment with iodine; *b*, micrococcus; *c*, *Spirochæte dentium*, after iodine; *d*, *Spirillum sputigenum* (× 800).

Spirochæte plicatilis.—If we have water in which Algæ, especially *Spirogyra* and *Vaucheria*, are decaying, and examine a little of this fluid, we shall find in it, almost to a certainty, motile, exceedingly fine spiral threads (Fig. 103) like those already found in the fur of teeth, but much longer than they. These flexible corkscrew-like threads move rapidly in the water. They turn on their

axis, and at the same time bend to and fro. Individuals suddenly stand still, then hasten on again. The spirals found under such circumstances in all probability belong to *Spirochæte plicatilis*, the *Spirochæte* of marshes. If these spirals are allowed to dry, and are stained, we shall see that they are not unicellular, but consist of successive segments, which may vary in length according to circumstances.—Very fine *Spirochæte* can usually be obtained by keeping in a warm place a filtered infusion of pea-flour.

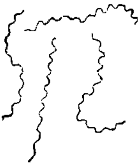


FIG 103. — *Spirochæte plicatilis*, after aniline staining, partially showing the segmentation into rodlets (× 540).

Pure Cultures. Bacillus subtilis. — We will now endeavour to follow without a break the developmental history of a bacteriad, selecting the hay bacillus for the purpose. We first of all soak some dry hay in the smallest possible quantity of spring water, and let the infusion stand for four hours in a warm chamber at a constant temperature of 36° C.[1] We then pour off the infusion, without filtering, and dilute it down, if too strong, to a specific gravity of 1·004, or a pale sherry colour. Now place the fluid in a flask holding at least 500 c.cm. The flask is stopped with a plug of cotton-wool, and the fluid boiled very gently for an hour. Then let the temperature sink to, and remain at 36° C.[1] In the course of a day, or a day and a half, a delicate grey skin, the pellicle, will have formed on the surface of the fluid; this consists of the *zooglœa* stage of *Bacillus subtilis*, the bacteriad of hay. We have made use of the power possessed by the spores of this bacteriad, of resisting boiling heat for a considerable time, in order to obtain a pure culture of them. —Of the pellicle obtained as above we now transfer a little, with the platinum loop, to an object-slide, and examine the object with the strongest magnification which we have at command. We find the pellicle formed of long, segmented, wavy threads, running parallel to one another. The threads remain for the most part in position, because they are held together by an invisible jelly (Fig. 104, *A*). The threads consist of cylindrical rodlets of various lengths—in general, however, twice or thrice as long as broad. The substance of the threads appears homogeneous, colourless, pretty highly refractive. Even with the strongest magnification no other structure is recognisable. With chlorzinc

[1] This temperature, though advantageous, is not essential. A warm room temperature will suffice, but the action is slower.—[ED.]

iodine the rodlets are stained throughout, and very clearly, a brownish-yellow. The figures are thus obtained better than with the other solutions of iodine. The segments of the threads then appear in general shorter than in the fresh state, because all the limits are now visible. In order sharply to differentiate the rodlets we can stain them, according to the methods already known to us, with fuchsin, methyl violet, gentian violet, or Vesuvin, and then keep them as permanent preparations in Canada balsam or in dammar.

If we focus upon some particular spot in the preparation of pellicle with a magnification of about 1000, we can observe the division (segmentation) of the rodlets direct. It is best to draw the piece of the thread in question at short intervals with the

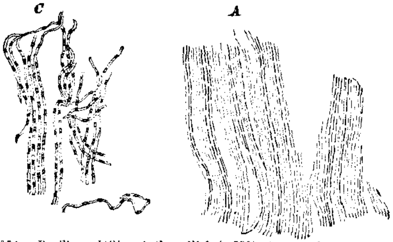


FIG. 104.—*Bacillus subtilis*. *A*, the pellicle (× 500); *C*, spore-formation (× 800).

camera, and compare the drawings, so as to show the changes which have taken place. If abundant food-stuff is still in the fluid, the individual rodlets divide in every half-hour to an hour and a half. The higher the temperature of the room, the more rapid the division. The rodlets increase in length without becoming thinner; when they have attained, however, a definite size, a dark-looking partition wall appears across their middle. This process of division explains the arrangement of the rodlets and threads; it explains also the wavy course of the threads, which grow at all points by intercalary growth, and, if the ends cannot become farther removed, the thread must become laterally contorted. For this reason, the whole pellicle shows a wrinkling, visible to the naked eye.—We next transfer a fragment of the pellicle into a moist chamber, in order to examine it in a sus-

pended drop. For this purpose we will use the simplest possible moist chamber—to wit a small frame of pasteboard. Such a frame is cut out of tolerably thick pasteboard, its inner aperture being somewhat smaller than the size of the cover-glass we propose to use, while its outer diameter does not exceed the width of the object-slide. This frame is soaked in distilled water till it is completely saturated, and then laid upon an object-slide.[1] On the middle of a cover-glass, which has been sterilised in a flame, is placed a drop, spread out very thin, of the culture fluid, into which the object for investigation is transferred. The cover-glass is turned rapidly upside down, witb the drop hanging below, and laid upon the pasteboard frame. If tbe observation is to be long continued, a few drops of water are from time to time placed upon the frame, so that it shall not become dry. If the observation is interrupted, the preparation can be protected from evaporation in a large moist chamber composed of a bell-jar standing on a large plate, with its edges immersed in water on the plate. In order to again find a definite spot in the preparation, it is well to cut a cross on the stage by means of a sharp instrument, rigbt and left of the central aperture. Then, when the object-slide is in the required position, similar crosses can be made upon it with one

[1] After and before using the pasteboard culture-cell it is desirable to place it for a few minutes in boiling water, so as to kill any organisms which may adhere to it, or the culture may be vitiated. This applies equally strongly to other cultures.—If cultures are to be at all prolonged the pasteboard cell is preferably replaced hy one made out of a glass ring. These glass rings, about ⅛-inch high, are cut from glass tubing of suitable diameter, and have their cut surfaces flattened quite horizontally upon a whetstone; and are then fixed upon the object-slide with Canada-balsam. A drop of water is placed at the bottom of the cell to keep the air saturated, and a cover-glass of suitable dimensions, after preparation of the hanging drop, is fixed upon the glass cell by oil-drops run round its edge. Glass rings of this kind can be purchased, ready cut and smoothed. Such a glass cell can be converted into a **gas-chamber**, for the purpose of testing the influence of various gases upon living organisms, by having openings in it opposite to one another, in which small glass tubes can be cemented or fused, and the gas then drawn through by an aspirator. An admirable gas-chamber is that designed by Prof. Marshall Ward, and figured in *Phil. Trans.*, 1892, vol. B, p. 131.—Object-slides bearing moist-chamber cultures may be placed in a **plaster of Paris case**, made with a removable plaster lid. This will serve as an admirable moist-chamber for the culture of fungi and bacteria, which do not need light, since the moisture from its damped walls, or a water reservoir at the bottom, is very uniformly distributed, and no drops of water can fall from above upon the preparation. These cases are very easily made, and of any desired size.—[ED.]

of the sharply-pointed colour pencils (Faber's) mentioned in the Introduction, or with a writing diamond. It is then easy later on to replace the object-slide thus marked in exactly the same position.[1] If the food-materials of the drop are approaching exhaustion the vegetative segmentation or bipartition is arrested, and the endogenous spore-formation at once begins. After the lapse of from six to eight hours there can be seen in the threads, at thereabouts equal distances, elliptic, strongly refractive spores (*Fig. 104 C*). *The threads appear otherwise empty; only* the colourless sheaths unite the spores. At various places in the preparation, one is certain to find spores still in course of formation. They appear in the form of collections of more refractive material, situated most usually towards the middle of the rodlet. The aggregation becomes continually more marked, while the rodlet becomes emptied, and at last the formation of the spore is complete. If the culture is allowed to continue some hours longer, the sheaths of the rodlets will have become indistinct, and after the lapse of about a day the spores appear free, and sunk to the bottom of the drop.—Under unfavourable cultural conditions, as, *e.g.*, with too large a proportion of sugar in the culture fluid, *there arise irregular swellings and abnormal changes of form in* the cells (involution forms).

The spores germinate very easily if they are transferred to fresh nutrient fluid; more slowly at the temperature of the room, quicker at 30° C. (= 86° F.). It is best to boil them for five minutes, *and cool them slowly.* Then in about two to three hours we can see the commencement of germination. The spore-membrane is opened on one side, the minute germ begins *to protrude here, and elongates gradually into a rodlet; so that* the long axis of the sporeling is at right angles to the long axis of the spore. Its hinder end remains inserted in the spore-case. About twelve hours elapse before the rodlet divides for the first

[1] For the purpose of keeping a particular spot under observation for several successive days, *i.e.*, without removal from the stage of the microscope, one end of a few strands of loose wick can be inserted between the layers of the pasteboard, while the other end can dip into a vessel of distilled water. The water sucked up by the wick will keep the pasteboard moist. Opticians also construct mechanical stages to microscopes, by means of which a movement in two rectangular directions is possible, an apparatus, which, with its appropriate "finder" not merely facilitates replacing an object in any exactly-desired position, but also enables a rapid survey of the whole of a large surface to be made.—[ED.]

time. Preparations examined in the interim unite all stages of germination. As a rule, the germinated rodlets at once set up movement; they enter into the "swarming" or "roving" stage. Such a swarming rodlet still carries about at its hinder end its spore-case. The number of the "swarmers" becomes by successive divisions continually greater, and they fill the entire fluid before the beginning of the formation of the pellicle. Then for the first time the swarmers collect on the surface of the fluid, come to rest there, and produce the pellicle. The rodlets are of unequal length, but for the most part consist of two connected individuals. Their movement is quite characteristic, flittering across the field of view.—Modern research has shown that the swarmers move by means of numerous cilia distributed over their body (Fig. 105).

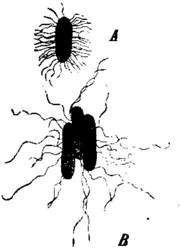


FIG. 105.—Swarmers of *Bacillus subtilis*, after Fischer (× 1500).

Bacterium termo.—The movement of the swarmers of *Bacillus subtilis* under a cover-glass soon ceases; and we may study this phenomenon more advantageously upon the so-called *Bacterium termo* (a general name rather than that of a particular species), the constant accompaniment of numerous processes of decay. We may reckon pretty well with certainty to find swarmers of this group in water in which peas or beans have been left to rot. We can readily prepare a suitable culture if we take a drop of this fluid and transfer it to a proper nutrient solution. For such, Cohn's "normal solution" can be recommended; this consists of 1 gram acid phosphate of potassium, 1 gr. sulphate of magnesia, 2 grs. neutral acetate of ammonia, and 0·1 gr. calcium-chloride dissolved in 200 c.cm distilled water. By repeated transfer of infected drops into new culture fluid, a quite pure culture may be obtained, if desired. In a few days the culture fluid becomes milkily turbid, and forms then a greenish pellicle. Microscopical investigation shows rod-like cells, of about 0·0015 mm. long, and from $\frac{1}{2}$ to $\frac{1}{3}$ that in breadth, in course of active bipartition, and thence united in pairs, rarely in longer rows. The movement is peculiarly jerky. Motionless individuals fill the zooglœa, which ultimately forms a greenish slimy skin or

clots on the surface of the fluid. — If now we examine the swarmers in a drop of fluid under a large cover-glass, it will be seen that the movement soon ceases. It is continued longest around any *enclosed air bubbles, and at the edges of the cover-glass*. Round the edge a thick layer of swarmers will soon have collected, cutting off all entrance of air. Ultimately all the swarmers come to rest. If, however, in making the preparation we have enclosed in it a green algal thread, the movement of the swarmers continues around *this thread so long as it is subject to the influence* of light. They collect in considerable number round the thread, and if this contains chromatophores confined to special parts, these parts are sought out by the bacteria. The oxygen given out by the chromatophores acts as a stimulus, which causes the movement of the bacteria, and determines even the direction of the movement. For example, in *Spirogyra* the aggregated bacteria follow the green band. If the preparation is placed in darkness, the movement ceases round the green cells also; and it recommences immediately the thread is again exposed to the action of light, whereby it begins to assimilate and give off oxygen. To prevent access of oxygen from the atmosphere a large cover-glass should be used, and this may even be sealed at its edges with paraffin or with cocoa-butter. The swarming stage of bacteria is, therefore, a very suitable test for oxygen, and it has been made use of in the case of **Engelmann's bacteria method,** in order to measure the strength of the assimilation of carbon in the different parts of the spectrum, and so in some degree to measure the relative values for this purpose of the various rays which constitute white light. Special micro-spectral objectives can be obtained for the purpose of throwing a small spectrum upon the object-slide under the microscope; or, if such a micro-spectroscope is not at our disposal, we can obtain a partial idea of the energy of the assimilation of carbon under the influence of different rays by allowing the light to pass through coloured glass, or coloured fluids, the spectroscopic character of which we have previously determined.

Culture Methods.—The greatest possible attention has been given to culture methods for bacteria, in consequence of the theoretical and practical importance which they have acquired. These cultures have become an important aid for the identification of bacteria, which can often be more readily distinguished macroscopically, by the form and method of growth of their colonies,

and often characteristic colour, than by microscopical means. For developmental purposes it is of the greatest importance to employ such cultures when information as to the relations of definite forms is required. Lastly upon such macroscopic cultures often depends the problem of determining the presence in certain media of spores capable of germination, and perhaps even to settle their number. The most important condition for all these investigations is that the culture shall start, and shall remain, free from any kind of accidental contamination. The problem how to carry on "pure cultures" has therefore assumed the highest importance.

To foster and perfect the methods of bacterial culture has now become the special duty of institutes for hygienic purposes. We will ourselves be satisfied with obtaining general information as to these methods; and only turn our attention more closely to them in so far as they may also have a bearing upon the botanical problems of bacteriology, and the further culture of the lower plants.

The rearing of bacteria was formerly carried on almost exclusively in fluid media; a transparent solid nutrient substratum was first brought into general use by Robert Koch. This solid transparent nutrient substratum is prepared from a gelatinous stiffening basis, to which one adds a suitable nutrient solution. Thus, according to the needs of the organism to be cultivated, the nutrient gelatine is prepared with hay-infusion, flesh-extract, peptone-solution, or blood-serum. In order to cultivate our hay-bacillus upon a solid nutrient material this latter would be prepared from hay-infusion and gelatine; for pathogenic bacteria a nutrient medium, to which is added peptone-sodium-chloride-bouillon, plays the chief part. The nutrient solution in this latter case is prepared from infusion of meat (beef-tea) with peptone and table salt, and made feebly alkaline with basic sodium phosphate or sodium carbonate. Without gelatine this nutrient fluid may be spoken of simply as a nutrient broth; if combined with ordinary gelatine it is called Koch's nutrient gelatine; with agar-agar, nutrient agar. Nutrient gelatines to suit his special purposes the botanist will have to prepare for himself; those used for pathogenic organisms, in the preparation of which special precautions are required, are better procured ready made. A considerable number of firms concern themselves with the preparation of such gelatines for micro-chemical use, amongst others that of Dr. C. Grübler of Leipzig (whose products can be obtained from Southall Bros. &

Barclay, Pharmaceutical Chemical Manufacturers, Birmingham, and G. Morton & Co., Importers and Agents, 19-21 Wilson Street, Finsbury, E.C.). Besides nutrient gelatine and nutrient agar-agar sterilised blood-serum is also necessary in many cases, and can be obtained from them. These nutrient bases can be obtained, as desired, in tubes, bulbs or flasks. Tubes are most convenient, and cost about 3s. 6d. per dozen.

Plate Cultures.—For the purpose of plate cultures, the gelatine is inoculated in the tube. For these purposes the tube of nutrient gelatine is warmed carefully, and not too greatly, over a flame, and when the gelatiue has become fluid, the plug of wadding is taken out. The rim of the tube is heated in the flame in order to kill any germs from the air which may cling there. The gelatine is then inoculated either with a straight or a looped platinum wire (previously sterilised in the flame) according to the quantity of material that one wishes to transfer to the gelatine; and with this wire the material is distributed as uniformly as possible in the gelatine. It is as a rule advisable not to be satisfied with this first inoculation, which is almost invariably too strong, but to prepare three tubes with the fluid gelatine, and after inoculating the first, to take three dips from it with the platinum loop and mix carefully in the second tube, and then take three dips from this second and mix with the gelatine of the third tube. Thus you have three quantities of gelatiue inoculated in three very different degrees. The three glass plates upon which the inoculated gelatine is to be poured out are placed for about a half-hour in a dry chamber at a temperature of 160° C.—the chamber being a box made of sheet iron and closed by a dust-tight lid; or the plates can be sterilised in a flame. We wait till the plates are cool and then lay them upon a larger sheet of glass which rests upon broken ice and water in a glass dish. Covered with a glass bell-jar the plates cool rapidly upon the ice, when the contents of the gelatine tubes, which in the meantime have been made fluid and inoculated, are poured out upon the plates. The plates should be 13 cm. long and 8 cm. broad; the edge should, for the space of about 1 cm., remain free from gelatine. The gelatine is distributed as evenly as possible over the plate by means of the rim of the tube, which can with advantage be again heated before pouring the gelatine out. The gelatine should set instantly. It is advisable to designate the plate which received the contents of the tube first inoculated with O; that with the first degree of dilution I; with

the second degree II. The plates are placed upon suitable shelves, under bell-jars, with observance of the needful precautions. In very accurate work, to prevent as far as possible accidental inoculations, the processes should be carried on in a quiet room, and as rapidly as possible. The gelatine upon the original undiluted plate O generally becomes turbid even after twenty-four hours at a room temperature. In the next few days the separate bacterial colonies become visible to the naked eye, not only upon the original plate, but usually also upon those which had received diluted material. Differences in the behaviour of the individual colonies soon arise, in their form, their colour, and the liquefaction of the gelatine, which either may not ensue, or may ensue in greater or less degree. In order to be able to examine the colonies upon such a plate with a high power, a cover-glass must be laid upon the part to be examined. If the cover-glass be subsequently raised, a "cast" of the surface colonies often clings to it, and such a "cast" can now be treated as a cover-glass preparation. Such preparations naturally cannot be expected till the gelatine has been liquefied by the bacteria.

Tube Cultures. — Plate cultures form the starting-point for tube cultures. From such a plate a portion of a definite colony can be removed with a platinum needle. The gelatine tube which is to be inoculated is held upside down, the wadding plug carefully withdrawn, and the platinum needle inserted upwards into the gelatine according to the purpose in view, either with a "prick" or a "scratch". In the prick inoculation it is stuck right into the nutrient gelatine almost to the bottom, in the scratch inoculation we make a shallow scratch on the surface of the gelatine.

It has been long determined that direct sunlight exerts an injurious influence upon bacteria, and often kills even their spores in a very short time. Hence bacteria cultures must under all circumstances be protected from this injurious influence.[1]

The macroscopic aspect of the cultures gives, as we have more than once said, many starting-points for distinguishing bacteria. The nutrient solution either remains clear, or it becomes turbid and forms clouds or precipitates of various characteristic appearances. The solution becomes diffluent, or becomes slimy, viscous, often changes colour and chemical reaction. This occurs still more notably in cultures upon transparent substrata. We

[1] See especially the researches of H. Marshall Ward in *Phil. Trans.*, vol. clxxxv., p. 961, 1895.

see spherical, regularly elliptic, clustered or otherwise shaped, and lastly also entirely irregular colonies. In all gelatine cultures it is necessary first of all to distinguish bacteria which leave the gelatine solid, from those which liquefy it. In tube cultures the bacteria of the first category soon form at the pricking point a small projecting boss, so that the channel of the prick together with this boss takes the form of a pin; or they spread upon the surface in the form of concentric rings or foliaceous or grape-like figures; or they grow more within the prick itself; or radiate from this into the surrounding medium. The colour of the colonies is very various, and often the gelatine also takes on a definite colour, or changes in some way or another its appearance. Characteristic odours often accompany all these processes

Just as for cultures in gelatine tubes, the gelatine plates can be used as starting-points for cultures in suspended drops, in moist chambers, or also upon certain sterilised solid nutrient substrata. For these latter, disks of potato play the chief part. As these show a slightly acid reaction, it is desirable before sterilising to lay them for a short time in a 1 per cent. solution of soda. The potato disks are best sterilised in a somewhat tapering tube, in which they cannot go to the bottom and therefore cannot be exposed to the action of any condensed water. Sterilisation results from exposure for fifteen or twenty minutes in a steam chamber, or a culinary "steamer" with a vent pipe for steam makes a fair substitute. Just as in sterilising gelatine, the tube can be closed with a *cotton-wool* plug. *In order in these manipulations to avoid contamination from the hands, it is desirable first to wash these thoroughly with soap, and then to disinfect with 1 per cent. solution of corrosive sublimate, and also* even to previously dip in sublimate solution the finger with which the objects will be touched.

After any bacteriological investigation it is very desirable to destroy all the material, so that it may not be a source of infection for other cultures; and the utensils used can be sterilised either by laying for several days in 1 per cent. sublimate solution, by boiling in water for half an hour, or exposure for a like time in the steamer.

Finally for the study of **pathogenic bacteria** inoculation experiments upon healthy living animals have the utmost importance, since by these means the infective properties of bacteria are first established. The infection is made, with all needful pre-

cautions to exclude contamination by alien germs, either cutaneously, or, more commonly, subcutaneously. The cutaneous inoculation is usually carried on upon places from which, like the ear, it cannot be licked off by the animal. In subcutaneous inoculation the skin, raised with forceps, has either a prick or an injection made. With the guinea pig the inner side of the upper part of the thigh, with the mouse the root of the tail is usually selected for the operation.

Life Histories.—In nearly the whole of our microscopical studies thus far, extending up to Chapter XX. of this work, we have concerned ourselves only with the morphological characteristics of vegetable organisms, proceeding from the more complex to those of extremely simple structure. In the group of the bacteria, however, we have departed from this line of study. Their morphological characteristics are so simple that in becoming acquainted with them we have at the same time come face to face with another set of phenomena,—have realised that each of these organisms has a **life history or life cycle.** In that life history or life cycle we at least realise the existence of two characteristics: that the organism, essentially unicellular, can nourish itself, grow, and the cell which constitutes it undergo segmentation, so that each organism gives rise to two daughter organisms, each of which is, practically, half of its parent; we have, that is, a method of **multiplication of organisms** of the most simple kind, which we have called "fission"; that, on the other hand, under certain circumstances many, at least, of these organisms can undergo a sequence of changes in which the active life of the individual is suspended, and its living substance is aggregated into a body, the **spore, or resting spore,** which is capable of passing into a period of rest, in which it can be dried and often exposed to great extremes of temperature with impunity; but from which state of rest it can be aroused by suitable conditions, and can then re-enter its ordinary phase of active, self-nourishing, growing and multiplying life. It goes as a matter of course that its nourishment is effected by means of the food substances contained in the material in or upon which it was grown, whether liquid or solid, and that these nutrient substances it manipulates in such a way as to extract from them the constituents of its own food. In this process it does not necessarily use up all the chemical bodies of which the nutrient substratum is composed,

and may carry on the manufacture of what we may call "bye-products". More than once we have referred to the development of characteristic colours, smells, etc., as products of the chemical activity of the bacteria. Actively concerned, then, as they are in the breaking down of organic chemical bodies, many of them carry on their nutrition by manifestly inducing changes which it has become convenient to speak of as "fermentative".

Life History of Yeast.—We will now make the acquaintance of another fungoid organism, likewise of very simple character, the whole life cycle of which we can study by means which our labours with the bacteria have made us conversant with, and in which fermentative action, of another kind however, is very strongly marked. These are the very simply-constructed fungal organisms hitherto collected together under the name of **Saccharomycetes.**

We provide ourselves with some **Yeast,** the "barm" used in brewing beer, and examine a trace of it, diffused in water, under a high power. We find the field of view filled with small cells, individuals of the so-called yeast fungus, *Saccharomyces cerevisea.* The cells appear globular or elliptic; they have a delicate membrane, and in the interior can be recognised a large or several small vacuoles, and some highly refractive granules. A nucleus can only be distinguished by the aid of hardening and staining, and then with difficulty, but there is no uncertainty as to its existence; while it is seen, also, to divide in the process of cell-multiplication. Numerous cells are seen in course of multiplication. This takes place in a quite characteristic and peculiar fashion, by the cells forming one, rarely more, small knob-like swellings, which gradually attain the size and form of the mother-cell, and then are separated from it by a partition wall. In very energetic development we find the daughter-cells united into small, occasionally branched, chains; in slower development the cells separate before any new one commences to form. This is multiplication by **budding,** peculiar to the Saccharomycetes. In sugar-containing fluids, yeast brings about **alcoholic fermentation.** The individuality of the Saccharomycetes has been much questioned, and they have been declared to be **conidia** (that is spores, of a kind), of different fungi, which have ex..he power, in a suitable nutrient fluid, of multiplying by budding anc..ndefinite sequence.

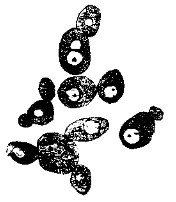


FIG. 106.—*Saccharomyces cerevisiæ*; budding and non-budding cells (× 1200).

irst ..he pure culture of yeast has now a very high technical ..nce. The great breweries work only with pure yeast, .. same is needed now a days for the fermentation of the

"*must*" in the preparation of wine. The methods of pure culture are essentially the same as for bacteria. A nutrient gelatine, prepared by dissolving from five to six parts gelatine in "wort" of beer (*i.e.*, infusion of malt), which has been "hopped," is recommended as a solid transparent medium for culture. We prepare a cover-glass with the nutrient gelatine, inoculate with the yeast, invert it upon a moist chamber, and determine by direct microscopical observation whether the colonies developing in the culture material are actually derived from single cells. In contradistinction to bacteria, no conclusion as to the nature of the yeast can be drawn from the habits or appearances of the flecks, since different species can have the same appearance, and different colonies from the same species can have differing appearances. From cultures controlled under the microscope yeast cells can be removed with the looped platinum wire, previously heated red-hot, and with them flasks containing sterilised wort, or other selected nutrient solution, can be inoculated.—For laboratory purposes yeast can be grown, either in bulk or in moist chamber cultures, in Pasteur's fluid. In slide cultures very beautifully-branched chains of cells can be obtained. The simplest way of preparing Pasteur's fluid is to keep dry in a bottle, very finely powdered and most thoroughly mixed, 10 gram. potassium phosphate, 1 calcium phosphate, 1 magnesium sulphate, and 50 ammonium tartrate, or larger or smaller quantities, so long as proportional. For use dissolve in the proportion 1 gram. of this mixture with 12 of sugar in 70 cc of water.

The Saccharomycetes generally hibernate in the soil, but the yeast of beer is only known in a state of cultivation. By suitable cultures they can be led to produce spores, a number of which appear in each cell. This is easily effected when sterilised plates of plaster of Paris, placed in a flat vessel so as to be half-immersed in water, and covered over with a bell-jar, have strained or filtered yeast smeared upon them. Kept at about 30° C., spore formation in *Saccharomyces cerevisea* may follow even in about twenty-four hours, but more usually in from four to eight days. Pieces of fireclay have also been recommended for the culture of yeast-spores. They have the advantage that they can be cleaned with a brush, sterilised in a dry chamber at 150° C., and are then again ready for use.—All yeasts, however, do not sporulate with equal freedom, so that failure with material derived from one source may be corrected by obtaining supplies elsewhere.

CHAPTER XXII.

THE REPRODUCTION OF ALGÆ.

Principal Materials Used.

Spirogyra in conjugation; fresh. Or, fixed with 1 per cent. chromic acid, and preserved in water containing a little camphor.

Cladophora glomerata; fresh.

Vaucheria sessilis, preferably from running water; fresh; or, if need be, the terrestrial form. For swarm-spore formation, *Cladophora* or *Vaucheria* should be collected a few days before wanted, and placed in flat saucers with water; sexual organs are produced in *Vaucheria* by cultivation for a week in 2-5 per cent. sugar solution, and bright light.

Fucus platycarpus (or *F. vesiculosus*, male and female), fresh; also in alcohol.

Chara fragilis; fresh.

Principal Reagents Used.

1 per cent. osmic acid—Iodine—Alum carmine—Logwood.

In our morphological studies we have followed the plan of commencing with the better-known highly-organised plants, and working downwards to those of the utmost simplicity. It is now our purpose to study microscopically the processes of reproduction; and here we shall follow the reverse route, ascending from the lowliest organisms to those of the highest degree of organisation. We have already commenced this study, in our last chapter, upon Bacteria and Yeast, to the entire cycle of development of which we have directed our attention. In the whole of this life cycle there was no indication of separate sexuality. The process of multiplication was vegetative, or asexual. Organisms of somewhat higher grade, however, show both of these processes, vegetative or asexual multiplication, on the one hand of entire organisms, or on the other hand of the constituent cells of those organisms, and the commencement of the life cycle of new individuals through more or less complicated processes of sexuality.

We will now proceed with the examination of examples of the asexual and sexual processes amongst the Algæ.

Conjugation of Spirogyra.—Opportunity often offers for examining the various species of *Spirogyra* in process of Conjugation.[1] This state is recognisable out of doors by the crinkled look (rather yellowish) and hanging together of their masses of threads, whereas the non-conjugating threads are dark green, and in open springy curves. The process can easily be followed. The threads should not, however, be directly covered with the cover-glass upon the object-slide, but the small pasteboard moist chambers described upon p. 279 can be used with advantage, the *Spirogyra* being placed in a drop suspended from the cover-glass. If covered direct, pressure of the cover-glass should be obviated by placing under its edges narrow strips of paper. Conjugation in most cases takes place in ladder-like fashion, *i.e.*, two threads lying alongside one another are connected by cross bridges (Fig 107 *A*). The cells put forth short blunt protrusions, which come into contact, and fuse with one another, forming the conjugating canal. The cause which brings about the formation of these processes and their union is a chemical stimulus. Curvatures can also be induced by the same means, by which threads not at first rightly placed can assume the correct position. In many cases we can distinguish prior to conjugation which thread is male and which female, since the cells of this latter sex swell out into barrel-form; but not infrequently this means of distinction is wanting. After the union of the conjugating processes the contents of the male cell first round off, and finally withdraw on all sides from the cell-wall. They then pass into the conjugating canal, and through the middle partition walls, which in the mean-

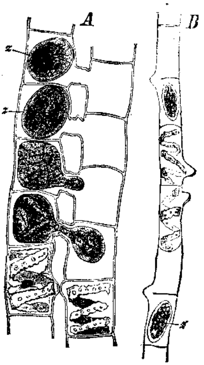


FIG. 107.—Conjugation in *Spirogyra quinina*. *z*, Zygospores (× 240).

[1] Fresh-water Algæ can be obtained from T. Bolton, Balsall Heath Road Birmingham; marine algæ from the Biological Station, Plymouth.

time had become softened. The female cell had either rounded simultaneously, or rounds off on entrance of the male cell. Both cells come into contact, and after a few minutes coalesce. Their contents blend. The **zygote** or **zygospore** thus formed begins at *once to contract*; *after the course of an hour its vacuole*, hitherto filled with cell-sap, has completely disappeared, the cell-sap being expelled. The vacuole is again formed about twenty-four hours later. A membrane with clear double contour then clothes the zygote (Fig. 107 *A*).

Thus much can be seen without reagents. If, however, the object is fixed and stained during conjugation, say with 1 per cent. osmic acid, and made transparent by placing in dilute glycerine, which is allowed gradually to concentrate by evaporation of the water, it can be determined that the two nuclei of the conjugating cells, after the fusion of these latter, gradually approximate and ultimately combine into a single one. The whole of one thread is emptied, the other contains zygospores; the former is male, the latter female; that is, the threads are unisexual. Of the chlorophyll bands only that (or those) of the female cell remains intact; that of the male is reabsorbed, and more or less completely disappears. The ripe zygospore acquires ultimately a thick membrane, in which several layers can be distinguished, of which the outermost and innermost are colourless, the median is brown. In the interior of the zygospore numerous oil-drops are produced, and flecks of a red or reddish-brown pigment.—*Occasionally the cells of a thread may conjugate* with either of two other threads; and in other cases two adjoining cells of the same thread may coalesce by a curved conjugating canal (Fig. 107 *B*) showing that threads can be **monoecious.**

Spirogyra which one has in cultivation (see p. 251) can be very readily induced to conjugate at any time between mid-February and the beginning of May, by placing it in 2 per cent. to 4 per cent. solution of cane sugar and in a sunny position; and the same end can be attained with plants in the sun, but in a very small quantity of water. If the alga has, naturally, a predisposition towards conjugation, this can be brought about upon the micro-slide in ½ per cent. Agar-agar jelly. As the threads cannot change their position in this, only those threads which lie suitably towards one another can put out their conjugating processes. The other cells must remain sterile, but after some

time, and especially if dilute nutrient solution is added, can resume their growth and division.[1]

The process of conjugation we have just studied is characteristic of the entire section of Algæ collected together as **Conjugatæ.** To this, besides the species of *Spirogyra*, so widely diffused in fresh water, belong also the almost equally widely-spread species of *Zygnema*, recognisable by two stellate chromatophores in each cell; and the elegantly-formed Desmideæ. Into close connection with these latter we may even bring the Diatomaceæ, in which typical conjugation is likewise present.

Reproduction of Cladophora—Swarm-spores.—The genus *Cladophora*, belonging to the **Chlorophyceæ** (the structure of which is already known to us) provides us with a right favourable object for the study of **swarm-spores** or **zoospores**; it is only to be regretted that they are not always inclined to their production. It is comparatively easy to obtain swarm spores of the marine forms, which we keep in a large vessel with sea-water; and amongst fresh-water forms, *Cladophora glomerata*, if taken from rapidly-flowing water, and laid about evening in a shallow vessel with a layer of water about ½ inch deep, will be usually found to have swarm-spores next day. The formation of these commences at the apices of the branches, and proceeds towards their base; hence all stages of development are easily found close together. We examine these in acropetal succession, and commence with a still unchanged cell. The structure of this is already known to us (p. 247). So much as is visible without reagents we soon recognise again: the polygonal, closely-crowded chromatophores, containing small, pale, starch granules, in part also having larger pyrenoids; the plasmic plates which traverse the cavity of the cell, and in part also contain chromatophores. If we pass now gradually from such a cell to such as are being transformed into **sporangia,** first of all a change of the colour of the contents strikes us; later on the chromatophores begin to arrange themselves into a net, so that the entire contents of the cell (which have increased in quantity with proportional diminution of the central vacuole, the contents of which become slimy, and may even be obliterated) appear divided into approximately equal polygonal sections. At one place, usually near the anterior end of the cell, and in terminal cells occupying the anterior end, the cell-membrane swells, and, as the result of its increase in volume, bulges into a papilla-like projection. The chromatophores

[1] On the artificially induced conjugation of *Spirogyra*, see note, p. 310.

now withdraw towards the interior of the polygonal sections, and these latter appear bounded towards one another by clear lines. The sections then begin to round off, and so partially to separate from one another. The colourless peripheral protoplasm takes no part in the differentiation of the chlorophyll-containing contents into individual sections, but is transformed into a colourless mucus, which plays a part in the evacuation of the **swarm-spores (zoospores).**

The evacuation of the swarm-spores arises from the pressure of the cell-contents upon the gelatinous papilla, which they break through. Individual swarm-spores may also remain behind in the sporangium. If the object is examined in a suspended drop, under the influence of light, the swarmers ultimately collect either at the side of the drop turned towards, or at that turned from, the window. These swarmers are not, however, amongst those most sensitive to light; they remain for a longer time scattered in the drop, move about in indefinite directions, and only gradually, as their motile energy diminishes, arrive at the edge of the drop, where they come to rest. They then round off, and surround themselves with a cell-wall. The swarm-spore is pear-shaped, with a red **eye-spot** placed laterally by the colourless or green anterior "beak". The eye-spot is most readily recognisable with the use of a substage condensor and the diaphragm wide open. With a little iodine these swarm-spores can be very readily fixed (Fig. 108). We recognise now two cilia upon each (in other species of *Cladophora*, even four), which arise from the tapering anterior beak of the swarm-spore. The swarm-spores move with the ciliate end forwards. In swarm-spores lying in a favourable position, the small nucleus is thoroughly recognisable, after treatment with iodine, lying in the anterior colourless end. Instead of iodine, 1 per cent. osmic acid may be used for fixation (see Fig. 108).

FIG. 108. — *Cladophora glomerata*. A swarm-spore fixed with 1 per cent. osmic acid. On the right-hand side *is the eye-spot.* The nucleus can be seen in the anterior half (× 1000).

These swarm-spores which we are studying are asexual, but in *Cladophora* still other, smaller, sexually differentiated swarmers or **Gametes,** are produced. These conjugate with one another, but have hitherto been observed only in the marine forms. The larger, asexual, swarm-spores have in these latter four cilia—the smaller gametes only two.

Reproduction of Vaucheria—Swarm-spores.—From amongst the acellular multinuclear **Siphoneæ** we select for examination the widely-spread *Vaucheria sessilis* in order to study the formation of its swarm-spores (zoospores) and also its sexual organs. We can obtain swarm-spores of *Vaucheria* at any time if algæ which have for several days been kept bright and damp are flooded with water, or if algæ which have been cultivated in 0·2 to 0·5 per cent. Knop's nutrient solution (see p. 251) in a bright position are transferred to pure water, or if cultures in water or in 0·1 to 0·2 per cent. nutrient solution are placed in darkness. If we examine the culture with a lens of considerable focal length we can easily recognise the first formation of the sporangia by the dark colour of the ends of the threads. If now we seize with the forceps, at their point of junction, a group of threads which appear to be in the desired condition, and transfer them, without allowing them to be bent, to an object-slide, we can study upon them direct the further processes of development, which, moreover, often go on undisturbed under the cover-glass, if only the object is protected from its pressure by thin sections of elder-pith, or horse-hairs, placed under its edges. If a sporangium is to be formed out of the end of a branch, contents rich in chlorophyll collect in this, and the end begins to swell club-wise. The sap cavity is constricted below the end (Fig. 109, *A*), and is soon separated off in the upper part as a spherical vacuole. The sporangium is then cut off by a partition wall, in the formation of which the chlorophyll-containing contents of the young sporangium, and of the rest of the sac, temporarily

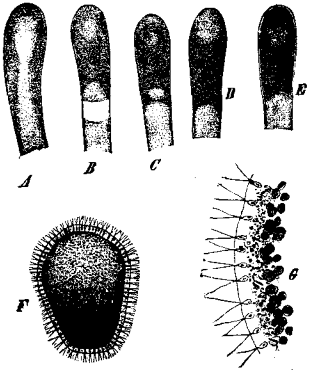


FIG. 109.—*Vaucheria sessilis*. *A* and *B*, formation of the sporangium; *C*-*E*, formation of the swarm-spore out of the contents of the sporangium; *F*, a swarm-spore set free; *G*, a portion of the outer, colourless plasmic layer (ectoplasm) from the anterior end of the swarm-spore. *A*-*E* (× 95); *F* (× 250); *G* (× 450).

separate from one another, so that we can see them separated by a clear interspace (Fig. 109, *B*). Around the contents of the sporangium is now formed a clear border (*E*), which soon shows radial striation. This border consists of colourless peripheral protoplasm, the radial structure arises from the numerous small elongated, radially-arranged nuclei, which are here collected (*F*, *G*). These nuclei show up clearly only after treatment with suitable reagents, and best after fixing with 1 per cent. osmic acid and staining with alum-carmine. They are only visible with a high power. It can be determined at the same time that from each nucleus arises a pair of cilia. The swarm-spore of *Vaucheria* is therefore **multinuclear.**—When the swarm-spore is fully formed, it is at once evacuated. The apex of the sporangium ruptures with a jerk, and at the same moment the anterior part of the swarm-spore issues from the opening, and simultaneously begins to rotate upon its axis. The swarm-spore has to squeeze through the opening. Its birth lasts usually somewhat over a minute, a swelling substance formed in the sporangium helping to expel it. In many cases, though comparatively seldom, the anterior end of the swarm-spore twists off from the hinder part, while still in the sporangium; the anterior part then hastens to form a complete, but correspondingly small, swarm-spore, and the posterior part forms a second swarm-spore. This is only possible by virtue of the multinuclear character of these swarm-spores, in that each half contains the nuclei necessary to its existence. The movement of the swarm-spore lasts about a quarter of an hour; the direction of the movement is not influenced by the direction of the rays of light falling upon it. The swarm-spore is ovate, broader anteriorly; in this anterior end lies the vacuole. Only in the moment when the swarm-spore comes to rest are the cilia visible; they cover the whole body as a short down. A moment later they are withdrawn into the body of the swarm-spore, which, during this process, shows a wrinkled surface. Afterwards the body again becomes smooth. During the withdrawal of the cilia it is noticeable that the swarm-spore has already surrounded itself with a very delicate membrane. The spore now rounds off slowly; its colourless border disappears, while the chlorophyll grains come to the surface; the cell-wall rapidly becomes thicker.

Sexual Reproduction of Vaucheria.—In cultures of *Vaucheria* …s, Vauch., sexual organs tend to be produced after some time; …t *Vaucheria* may be induced to form them in a few (say six

or eight) days if transferred to a 2·0 to 4·0 per cent. solution of cane sugar and exposed to bright light. In the variety *V. repens* the female organs, the **oogonia,** are usually placed singly upon the thread, each being accompanied by only one male organ, the **antheridium.** The oogonium is sessile on the thread, the antheridium terminates a short curved branch.

The **oogonium** is obliquely ovate (Fig. 110, *o*), densely filled with plasma containing chlorophyll and oil, and is separated from the thallus thread by a partition wall placed somewhat above the base of the oogonial branch. If an oogonium is observed at the moment of its origin, it will be seen that the contents of the thread withdraw from those of the rudimentary oogonium, in exactly the same way as in the formation of a sporangium (see *antea*). The oogonium is provided with a unilateral, beak-like outgrowth, in which colourless protoplasm is collected. In advanced stages of development this latter occupies the entire anterior part of the **oosphere.** If now we observe such an oogonium uninterruptedly, we shall see the colourless substance at the beak end put out a papilla-like projection, which rounds off more and more into an independent ball; this separates finally from the contents of the oogonium, and passes out into the surrounding water, where it slowly goes to the bottom. The membrane of the oogonium at the end of the beak is swollen, and the resulting jelly is ultimately completely dissolved. The remaining contents of the oogonium round off, its colourless apex is the **receptive-spot,** or place of fertilisation.

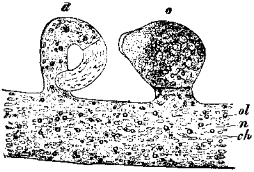


FIG. 110.—*Vaucheria sessilis.* Portion of the thallus with sexual organs. *o*, oogonium; *a*, antheridium; *ch*, chromatophores; *ol*, oil-drops. The nuclei *n* are represented, although they are only visible after suitable staining (× 240).

The antheridial branch is more or less strongly curved. Its bent end is formed into an **antheridium,** and is cut off by a partition wall (Fig. 110, *a*). In the ripe condition it is distinguished by its colourless contents, while the branch which bears it is rich in chlorophyll. The *antheridium usually turns its apex away from* the oogonium. In the colourless contents of the antheridium short rodlets, arranged longitudinally, are more or less clearly distinguishable. At the moment when the oogonium extrudes a portion of its colourless protoplasmic substance, the antheridium opens at its

apex, and evacuates its slimy contents, of which the greater part remains in the surrounding water in the form of colourless vesicles, which slowly disorganise; but a smaller part hastens away in the form of minute glancing **spermatozoids (antherozoids).** These actively swarming spermatozoids soon collect in the mass of jelly at the apex of the oogonium. Individuals press forwards to the colourless receptive-spot of the oosphere, and as it were grope around it. In specially favourable cases entrance of such a spermatozoid into the receptive-spot can be determined. After a short time the fertilised egg (the **oospore** or **zygote**) has surrounded itself with a delicate membrane, which is especially clearly visible at the receptive-spot. In the course of some hours the colourless protoplasm of the receptive-spot is distributed in the zygote. Older oospores are densely filled with oil drops, show some brown spots in the interior, and have a firm membrane.

If a swarming spermatozoid is fixed with iodine, two unequal **cilia**, unilaterally inserted, and extended in opposite directions, can be seen attached to it.

Reproduction in Fucus.—The various species of *Fucus*, or brown sea-weeds, found everywhere round our coasts, can be obtained in fructification nearly the whole year round. If gathered at high tide, when under water, or immediately after the setting in of the ebb, and sent damp, without any other packing, we are pretty certain to be able to observe the phenomena of fructification even at places far distant from the sea. The parcel ought to be accompanied by a considerable quantity of sea-water, or a suitable equivalent for this can be made from prepared salts. After receipt, a portion of the plant should be hung up free on a piece of string, the other part laid in sea-water. In about six hours, after the sexual organs have been emptied, the hanging plants can likewise be laid in sea-water; and, after about six hours, again taken out and hung up, and thus the evacuation of new sexual products induced. If the plants which were hung up immediately on arrival have not yielded sexual products, they may be expected from those which were laid in water, if these latter are taken out after about six hours and hung up to dry slowly. In cool weather the plants can stand a journey of even several days duration without injury, and by laying in sea-water periodically will develop normal sexual products for days.

Fucus platycarpus.—To study the structure of the sexual organs, we will choose in the first place the hermaphrodite species,

Fucus platycarpus, Thuret. This species is characterised by the production of male and female sexual organs in the same conceptacle. It is further distinguished from *F. vesiculosus*, which it otherwise closely resembles, in that it is always devoid of "air-bladders," while such are very general, though not invariable, in *F. vesiculosus*. Fertile specimens of both these species end their ultimate branchlets with bladder-like swellings. These bear the **conceptacles.** In *F. platycarpus* the swellings are stronger than in *F. vesiculosus*. Cutting sections of these swollen twigs offers some difficulty, on account of the strong tensions of the tissues, which result in the outer edges being folded inwards. The bladders collapse somewhat, while a part of the enclosed air escapes audibly. The interior of the bladder appears filled with a filamentous network, and partly also with colourless jelly. *Cross-sections* prepared between elder-pith show us that the tissue of the thallus has the same structure which we have already studied in *F. vesiculosus* (p. 240): outwardly the small polygonal cells of the epidermoid layer, inwards the progressively enlarging cells of the cortex, which elongate more and more, and ultimately pass over into the network of threads which constitutes the medulla or pith. The spaces between the threads are filled with jelly and air.

The **conceptacles** are pear-shaped hollows in the tissue. A narrow opening, the **osteole**, communicates with the exterior, and through this passes a tuft of delicate hairs. If the section has cut the conceptacle in the middle line, it is easy to get an idea of its structure. It is seen to be surrounded by a sheath consisting of several layers of closely-united, tangentially elongated cells. From the inner cells of the sheath arise numerous radially-arranged outgrowths, extending into the conceptacle, and reducing its cavity to a narrow cylindrical space, which diminishes as we pass outwards. In part these structures are **sterile hairs,** which remain unbranched. The number of these sterile hairs diminishes towards the upper part of the conceptacle. The cells themselves are elongated, many times as long as broad. The hairs close under the osteole, on the other hand, are composed of short segments. It is these hairs which protrude from the osteole in a *tuft.* The cell-contents include protoplasm, nucleus, and very small olive-green chromatophores. In structure much like these barren hairs, are the much branched hairs which bear the male sexual organs, the **antheridia.** The antheridia are unicellular branches of these hairs, have an elongated elliptic form and

abundant contents. Nuclei are not visible without special means; small chromatophores are numerous. In ripening, the contents collect into small balls, each with one, rarely two, reddish-brown chromatophores.

Between the sterile and the fertile hairs are found elliptic structures, the female sexual organs, the **oogonia**, which vary in size according to their stage of development, but ultimately attain to very considerable dimensions. The larger ones are coloured yellow-brown by small chromatophores; the abundant contents make them almost opaque, but with care it can be determined that they contain eight **oospheres**, with flattened contact surfaces. The smallest are unicellular, colourless in their periphery, transparent, with a brown fleck in the middle due to the aggregation of the chromatophores. Older stages show from two to eight such flecks, and finally thin cell-walls are formed simultaneously between these flecks, dividing the contents of the oogonium into eight fairly symmetrically arranged oospheres. After complete division, the brown colour is uniformly distributed through the contents of the oospheres. If the section has passed through the point of insertion of an oogonium, it will be seen that it is situated on a short unicellular stalk. In almost all sections through ripe conceptacles single oogonia will be torn off from their stalks. If such oogonia are observed for a time, an outer layer of the wall is seen to rupture at the apex, and the oospheres protrude, surrounded by an inner layer. This inner layer swells strongly in water, especially at its upper part, and gradually becomes diffluent and unrecognisable, and the oospheres are distributed in the surrounding water (Fig. 111, *E*). The oospheres round off, are devoid of membrane, and in the centre of each a clearer fleck is recognisable. If ripe antheridia are similarly broken off, after a while the contents are extruded, surrounded by an inner sheath (Fig. 111, *B*). After a time the contents escape in the form of small pear-shaped bodies, but in these cases movement is not usually seen.

Material hardened in alcohol cuts much better, and if stained with hæmatoxylin, gives beautiful figures, which amplify in not unimportant points the results obtained from fresh material. Thus, nuclei can be detected in the rudiments of oogonia, in number increasing, by repeated bipartitions, from two to eight; then follows the simultaneous formation of dividing walls, each oosphere having a central nucleus (Fig. 111, *D*). The nucleus

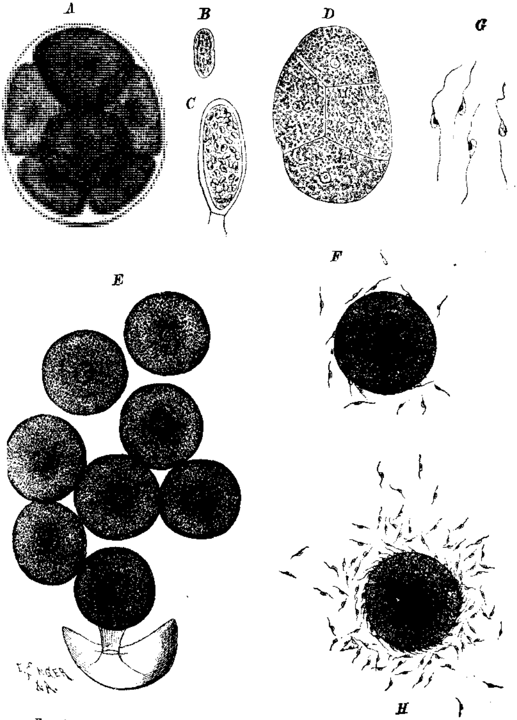


FIG. 111.—*A* to *F*. ***Fucus platycarpus.*** *A*, evacuated contents of an oogonium, surrounded by the inner cell-wall; *B*, evacuated contents of an antheridium, surrounded by the inner cell-wall; *C*, an antheridium, fixed with alcohol and stained with logwood; *D*, cross-section through the similarly fixed and stained contents of an oogonium; *E*, evacuated oospheres, and a remnant of the envelope of the oogonium; *F*, oosphere, with adhering spermatozoids.

G and *H*. ***Fucus vesiculosus.*** *G*, spermatozoids, fixed with iodine; *H*, oosphere, with adhering **and swarming spermatozoids. (*C* and *G* × 540; the other figures × 240).**

always corresponds in position with the brown fleck referred to above, so that we assume that each of these latter encloses a nucleus. The position of the nuclei is seen particularly clearly in the antheridia. In the ripening antheridium (Fig. 111, *C*) we can determine that almost the whole body of the spermatozoid consists of nuclear substance, which is surrounded by only a thin layer of cytoplasm. The whole of the protoplasm is not thus used up—a little, not staining with logwood, is left between the spermatozoids.

As a fixing reagent, and to some extent better than alcohol, can be used 1 per cent. osmic acid, picro-sulphuric acid, boiling water, or bromine vapour. Fixing with bromine vapour and boiling water often gives the best results, and is especially advantageous in that it does not require a subsequent washing of the preparation. The preparations can afterwards be stained with logwood, saffranin, gentiana-violet, or carmine, for particulars of which see *Cladophora*, *Spirogyra*, and "Cell-division". The stained preparations are then dehydrated and decolorised by 50 per cent. alcohol, which is gradually replaced by absolute, then cleared with oil of cloves or oil of marjoram, and mounted in Canada balsam or dammar.

In the plants left hanging in free air, after some search, will be found sexual organs which have escaped from a conceptacle. They appear as small olive-green drops of mucilage at the mouths of the conceptacles, and in which the oospheres can be seen even with a lens. If a little of this mucilage be removed with a needle, and placed in a drop of sea-water on a slide in a moist chamber, we shall see numerous oospheres and spermatozoids still enclosed in the inner sheaths of the sexual organs, and which repeat the phenomena given above. Soon, however, the antheridium-cases commence to empty, at one, or, less often, at both ends. The spermatozoids (Fig. 111, *G*) can commence to move before, upon, or not for some time after, evacuation. The movement is very active, and may last for several hours, but usually for much less. They are pear-shaped, with two unequal cilia, the shorter one attached to the anterior or pointed end of the spermatozoid, and directed forwards, while the longer one is attached laterally, and directed backwards. The reddish-brown fleck or eye-spot is at the point of insertion of this posterior cilium. The spermatozoids can be well and quickly fixed with iodine. Freed oospheres may be seen surrounded by swarming spermatozoids,

many of which cling to their bare surface (*F*). They attach themselves obliquely by the pointed end and a portion of the long side, so that the hinder cilia remains free, and for some time can be in motion.—If a sufficient number be present, they can give to the oosphere a rolling motion. This phenomenon is noticeable in the processes of fertilisation of various sections of the animal kingdom, *e.g.*, in the Echinoderms, the Actinia, and Vermes; but is known amongst plants nowhere else than in the Fucaceæ. The movement lasts from ten to twenty minutes, then the oosphere comes to a rest; a spermatozoid has penetrated and fertilised it. A cell-wall is then formed around the fertilised oosphere, or **oospore**. If fertilised oospores are kept in a watch-glass with sea-water, at the second, or, latest, the third day, the first divisions of the oospore can be made out. Unfertilised oospheres usually quickly become disorganised, without covering wall or division.—As antheridia and oogonia are found in the same conceptacle, fertilisation of the oosphere by spermatozoids of like origin can often result; but, nevertheless, fertilisation by spermatozoids from remote conceptacles is by no means excluded, and may be facilitated by the fact that the spermatozoids are usually evacuated before the oospheres of the same conceptacle, and often when the latter are evacuated the former are already swarming.

Fucus vesiculosus.—For the study of the sexual processes, *Fucus vesiculosus* is still more favourable than *F platycarpus*, and it is even more common. The structure of the sexual organs is much as in the latter plant, but one sex only, either antheridia or oogonia, is found in the conceptacles of any plant (**diœcious**). Plants hung up empty their sexual organs after a few hours. The drops of mucilage which contain the spermatozoids are recognisable, even to the naked eye, from their orange-red colour, while those which contain the oogonia are coloured olive-green. If a little of the orange-red mucilage is placed in a drop of sea-water, this will usually be seen almost immediately to be filled with actively moving spermatozoa. Thoroughly healthy spermatozoa are fairly sensitive to light, and nearly always avoid it (*i.e.*, are negatively heliotropic, or apheliotropic), so that even with slight illumination they usually collect at the room side, rarely at the window side, of the drop. With strong light their movement is fairly rectilinear, in the direction of the incident rays. Single spermatozoa from time to time stop suddenly, and

move for a little while in the opposite direction, but ultimately they all get to the shaded side of the drop. With very weak illumination a definite direction to the movement is hardly recognisable, and the same is the case with unhealthy spermatozoids. As the oospheres are specifically heavier than sea-water, the apheliotropism of the spermatozoa takes them away from the surface of the water, and therefore in the direction in which the oospheres are likely to be. The spermatozoa can be best fixed with iodine and picric acid, and show the same structure as those already studied (*G*).

In order to see the processes of fertilisation, we transfer the olive-green mucilage from the female conceptacle to drops of sea-water on a number of object-slides. We examine these in order to determine when evacuated oospheres are present. Such ought always to be found within the first hour, but oospheres which have been evacuated for several hours are still capable of fertilisation; so that we place our preparations in a dark chamber, and can use them for observation one after the other. If we follow under the microscope the method of evacuation of the oospheres, we shall see, in contradistinction to *F. platycarpus*, that the envelope of the oogonium remains visible up to the setting free of the oospheres, that its inner layer is specially clearly marked, and that during the evacuation of the oospheres the outer layer is turned over. If we now bring a little of the orange-red spermatozoidal mucilage into a preparation containing freed oospheres, these are quickly surrounded by spermatozoids. By turning the preparation in such way that the spermatozoids, avoiding the light, come into contact with the oosphere, we can determine that even those spermatozoids which are removed from the oosphere by a distance equal to the diameter of these latter, suddenly turn from their path in order to move towards the oosphere. This attraction extends over approximately twice the diameter of an oosphere. As has been determined by Pfeffer, this attraction depends upon a body given off from the oosphere, which acts as a chemical stimulus in determining the direction of movement of the spermatozoids. The spermatozoids cling to the oospere, which is soon completely covered with them. The spermatozoids lie obliquely on the surface of the oosphere, with the beak and a portion of that side which is devoid of cilia; while the hinder, laterally inserted, cilium continues for a time to vibrate, and sets the oosphere in rapid rotation. The rotation takes place

in the direction in which the beaks of the majority of the spermatozoids are directed; should the direction of rotation change, it is due to the attachment of new spermatozoids, thus altering the orientation of the majority. The movement is no doubt the resultant of the component movements of the spermatozoids; but if the movement tends to maintain any uniform direction, the spermatozoids arranged in any other direction tend gradually to alter their position, and to assume one corresponding with the movement. In addition to the attached spermatozoids, the oosphere appears to be surrounded by a swarm of free spermatozoids, which move within its sphere of influence (Fig. 111, *H*). In from ten to twenty minutes the rotation ceases, and there is no doubt that **fertilisation** has been effected, by the absorption of a spermatozoid; although, owing to the opacity of the oosphere, it cannot be observed. The **fertilised egg**, or **oospore**, has likewise developed an exceedingly delicate membrane. If a quantity of oospheres are mingled, as above, with spermatozoids, either on a hollow object-slide or watch-glass, and, after a few minutes, fixed and stained with iodine solution, we may be able to see the results of fertilisation. In most of the oospheres two nuclei can be seen: a larger one, with large nucleolus, the **oo-nucleus**; and a usually somewhat smaller one, with smaller nucleolus, representing the **spermo-nucleus**, or nucleus of the absorbed spermatozoid. The penetration of the spermo-nucleus towards the centre of the oosphere must take place very rapidly, for we find it commonly already in the neighbourhood of the oo-nucleus. In some of the oospheres the two nuclei have already been combined into a single one, in which process lies an essential characteristic of **fertilisation**. The **embryo-nucleus**, the result of the copulation of the oo-nucleus and spermo-nucleus, shows at first two, but later on only one, nucleolus.

Reproduction in Chara.—The small family of the Characeæ, or Stoneworts, occupy a somewhat isolated position in the vegetable kingdom, but are best placed amongst the green Algæ. Their structure, both vegetative and reproductive, is unique. They have a jointed stem, at the nodes of which leaf-like whorls of branches are borne. The internodes between two whorls are unicellular (as is noted on p. 246), and are either bare, or covered with a kind of cortical sheath of cells. The nodes are multicellular disks, bearing the branches on their periphery; from the nodes also proceed the cells which form the cortical invest-

ment of the internode. From the lower nodes arise the long, branched, obliquely septate rhizoids. The branches and so-called "leaves" are, in the main, repetitions of the stem. The sexual organs are produced laterally at the nodes, and we will study them in the widely-spread species *Chara fragilis*, which fruits freely in early summer.

Antheridia of Chara.—The **antheridia** of *C. fragilis* are recognisable even with the naked eye as red globules, with a

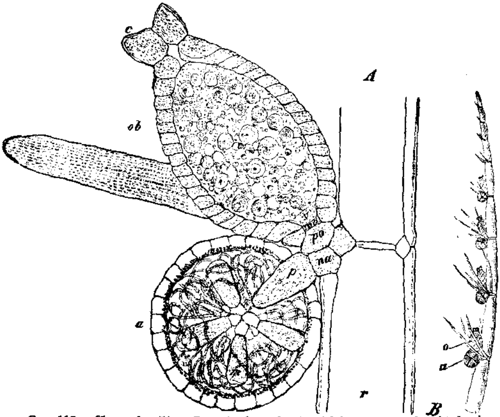


FIG. 112.—*Chara fragilis.* *B*, entire lateral axis with branches of the third order at the nodes, at each of the lowest four of which stands an antheridium (*a*), and oogonium (*o*) (× 6). *A*, median longitudinal section through a lateral axis *r*, and the sexual organs arising from it; *a*, antheridium, of which *na* is the basal node; *p*, the stalk and *m* the manubrium; *ob*, an oogonium or nucule, of which *po* is the stalk-cell, *no* the nodal cell, *v* the "Wendungszelle," and *c* the crown (× 90).

diameter of about $\frac{1}{50}$ of an inch. They stand singly, turned downwards, on the inner side of the branches, in their middle line (Fig. 112, *A*, *a* and *B*, *a*), and occupy the position of a twiglet. The female organ, the **oogonium**, or **nucule** (Fig. 112, *A*, *ob* and *B*, *o*), is found singly, close above the antheridium, and arises from the lowest node of the same twiglet which is developed into an antheridium. The antheridia have a complicated structure.

In order to realise it, let us examine a ripe antheridium externally with a magnification of about 100 diameters. It shows then apparently a red centre, surrounded by a colourless sheath. This colourless sheath is segmented by beautifully-arranged partition walls.—Now search for an antheridium which is as ripe as possible, and which will be found somewhere upon a branch, the uppermost antheridium of which (which first dehisces) has already fallen to pieces; cut it off with the needles, and crush it carefully under a cover-glass. *If the antheridium is thoroughly ripe*, its wall falls into regular pieces, the **shields**. From the interior come numerous long delicate filaments, and between these some cylindrical orange-coloured cells. These last are set into the centres each of one of the shields, like handles, and are known as the **manubria**, and their colour arises from elongated chromatophores. More careful examination further shows us that each such unicellular manubrium bears at its narrower end a colourless rounded cell, the **capitulum**, from which arise a number of smaller colourless cells the **secondary capitula**. From these arise the numerous slender, colourless filaments. Even with a power of 200 diameters we can see that each of these threads consists of a large number of flattened cells, forming a linear series. If the antheridium is ripe, we can see in each of these cells a coiled thread—the **spermatozoid**.

We now place our preparation in a suspended drop in a moist chamber, while we endeavour in another fashion to obtain information as to the structure of the wall of the antheridium. When the antheridium is crushed, a clear insight into the structure of this wall is hardly to be obtained; for the natural dehiscence of the antheridium upon the object-slide we should wait *in vain*; here and there, however, where antheridia have opened a short time before, the segments of the wall can usually still be found. They adhere to the branch, held together by the disorganised remnants of the threads. We separate one out with the needles, and can now easily study their form and structure. They are either triangular, or irregularly quadrangular. These segments, or shields, are flat, and are traversed by partition walls, which, directed towards a common central point, do not however reach it. Each shield is therefore unicellular, chambered, however, round its margin by the projecting ridges. The ridges correspond with indentations of the margin. The shields contain red globular chromatophores, which, separated by the ridges into

striæ, come together only in the middle of the cell. They lie close to the inner wall of the shield; whence it came that in examining the entire antheridium a dark red centre appeared to be surrounded by a clear sheath.

If we examine now a young but fully-formed antheridium, we can determine that the shields fit together by the indentations of their margins, and that eight shields, to wit, four above and four below, are combined in the wall. The four upper have the form of triangles, the four lower are quadrangular, because an angle of each of these latter is cut off, leaving a short side by which it joins on to the stalk of the antheridium.

A complete insight into the structure of the antheridium can however only be obtained by means of *sections*. To obtain these is not so difficult as might at first sight appear. A branch covered with sexual organs is placed between the fingers, the sexual organs turned inwards, and it is then halved longitudinally with a sharp razor. As a rule this suffices, but we may try to further divide one of the halves, so as to obtain a *median longitudinal section*. If this has been effected, we obtain the structure as shown in the annexed figure 112, *A*, *a*. The insertion of the antheridium (*a*) in the branch is clear. The stalk of the antheridium (*p*), with its wall lined with orange-red chromatophores, projects right into the interior of the antheridium. From the centres of the shields spring the *manubria* (*m*); the *capitula* seated thereon impinge upon one another and upon the stalk-cell. From the secondary capitula we can see proceeding the threads of mother-cells of the spermatozoids.

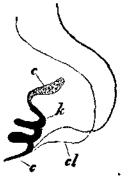


FIG. 113.—A spermatozoid of *Chara fragilis* fixed with osmic acid vapour. The darker portion *k* contains the nucleus (× 540).

Now let us again take the preparation with the crushed antheridium, which we had laid on one side. If this had been quite ripe, the spermatozoids will now, after the lapse of an hour or two, have commenced to free themselves from the threads. They emerge from their mother-cell by a lateral opening, showing brightly through a swelling substance which has been squeezed out from it. They are corkscrew-like threads (Fig. 113), with four complete turns. At a little distance from their anterior, somewhat tapered, end they bear two very long cilia, longer than the entire body of the spermatozoid, and readily recognisable after

treatment with iodine. At their hinder end the spermatozoids are somewhat thickened, and enclose glistening granules. The spermatozoids progress by simultaneous vibratory movement and rotation around their axis. The whole preparation swarms with spermatozoids, since the number produced in a single antheridium has been estimated at about 30,000. The antheridia tend to open spontaneously in the early morning. The spherical curvature of the shields diminishes notably, so that they separate from one another, and this tendency occasions the springing open of the organ. The spermatozoids are wont to swarm for some hours.

Oogonia of Chara.—In order to learn the structure of the **nucule** or **oogonium,** we preferably first study it in those stages of development in which it is still cylindrical and transparent. The antheridium found under it is then fully formed, and the oogonium itself begins to be somewhat brownish. In such an oogonium can be seen an elongated **central cell** or **oosphere,** densely filled with finely granular protoplasm. It is borne upon two flat internal cells, of which the upper is distinguished as the "Wendungszelle" (*v*), and the under is a nodal cell (*no*), and upon a short stalk-cell (*po*). This last is seated upon the nodal cell (*na*) which bears the antheridium. The central cell of the oogonium is sheathed by five tubular sacs, which arise from the nodal cell (*no*). These sacs run spirally around the central cell, and end above in the so-called **crown** (*c*). The five cells of this latter are cut off by partition walls from the investing sacs. If the crown consists thus of but five cells, we can at once determine our plant to be *Chara,* while the other genus of Characeæ, *Nitella,* possesses a ten-celled crown, consisting of five pairs of superposed cells—the result of a subsequent division of each of the five cells. In the investing sacs of the sheath of such young oogonia, **protoplasmic streaming** can be very beautifully seen. The chlorophyll grains have already elongated and taken on a brown tone. In subsequent stages the oogonium becomes oval, and the central cell, the egg-cell or oosphere, becomes filled with oil-drops and starch grains (Fig. 112) becoming thus opaque; the starch grains show a beautiful concentric lamination. The investing sheath becomes darker, and masses of lime become deposited on its outer surface.

Fertilisation.—The egg-cell is **receptive,** *i.e.,* is ready for fertilisation, at the same time when the antheridium of the same

branch opens. The tubular investing sacs close under the crown elongate a little, by which the outer layers of membrane of the sacs are torn at this spot. As the result of this we see the sheath which previously had been incrusted with lime right up to the crown, now become free from lime just under the crown. Simultaneously with their elongation the investing sacs have also separated from one another, and thus clefts arise which reach right into the interior, to the apex of the oosphere. By elongation and lateral separation of the investing sacs, therefore, a short "neck" has arisen under the crown, rendering fertilisation of the oosphere possible. If, indeed, we examine in the early morning the oogonia found in the immediate neighbourhood of the last opened antheridia, we shall find in and upon the clefts of the neck numerous spermatozoids clinging to them. They are arrested here by a jelly-like substance. The fertilised egg, or **oospore**, becomes surrounded by a strong colourless membrane, and the inner wall of the sheathing sacs bounding upon this commences after some time to thicken and become brown. In spite of the incrustation of lime, these relations can be made recognisable by treating the oogonium with hydrochloric acid.

NOTE ON THE CONJUGATION OF SPIROGYRA (p. 292).

Where conjugation is induced by placing in the sun in a very small quantity of water, there may be development of a parasitic fungus, *Vampyrella*, allied to the Chytrideæ. This does not appear in the sugar method suggested.

Nutrient solutions, even though dilute, retard or arrest the formation of zygospores. This retarding action is manifest even with a 0·1 per cent. Knop's solution.

Formation of spores by arrested development of the zygote.—When the surrounding conditions are unfavourable, the two conjugating protuberances may not come into contact. In this case the two gametes may round off separately, each become surrounded by a membrane, and germinate later on like a zygote, which they resemble in form and colour. We have then a case of **apogamy**; and the possibility of its occurrence suggests that the sexual differentiation of the gametes is very feeble. The same conclusion is suggested by the monoecious conjugation referred to on p. 292. Spores formed in this apogamic way are called **azygospores.** The formation of azygospores can be induced by 4 per cent. to 6 per cent. solution of sugar. Vegetating threads placed in agar-agar in the sun produce at the same time normal zygotes and azygospores.

CHAPTER XXIII.

THE REPRODUCTION OF FUNGI.

PRINCIPAL MATERIALS USED.

Mucor Mucedo, on fresh horse-dung, kept for a few days in a moist chamber
Phytophthora infestans, from diseased potato leaves.
Pythium de Baryanum, on cress seedlings.
Penicillium crustaceum.
Mucor Mucedo and *Penicillium crustaceum* are readily obtained, the former after a couple of days, the latter in about a week, upon damp bread, kept in a warm room under a bell-glass.

PRINCIPAL REAGENTS USED.

Iodine—Logwood.

WE will now study a few typical examples of reproduction, taken from the group of the Fungi.

Mucor Mucedo.—If a piece of damp bread is placed under a glass bell-jar, it is covered, even in a few days, with a thick felt of fungus threads (**mycelium**), which almost always belongs to the pin mould, *Mucor Mucedo*, one of the **Phycomycetes.** This fungus soon shows itself very luxuriantly upon fresh horse-dung, kept in a closed moist chamber. From the substratum arise erect fruiting branches, the **gonidiophores,** an inch or more in height, which bend towards the source of light, and end each one with a globular, yellow or brown, head, readily visible with the lens, and even with the naked eye. If we lift some of this material carefully from the substratum, and place it in a drop of water, we can determine, by means of sufficiently strong magnification, that the mycelium consists of thick, much-branched, irregularly septate sacs (**hyphæ**), and that from these arise the straight, unseptate and unbranched gonidiophores, each of which bears a globular head, the **gonidangium.** If still unripe, this remains unchanged in water; its contents consist

of yellowish-brown protoplasm. In the youngest stages the gonidiophore is not cut off from the gonidangium; later on there arises a partition wall, strongly arching into the interior of the gonidangium, so that the stalk ends within this latter with a swelling like a ninepin, the so-called **columella.** The ripe gonidangium deliquesces in water, and of its wall only fragments *formed of fine needles remain behind, of which it has been* determined that they consist of oxalate of lime. The expelled **gonidia** lie at fairly regular distances from one another, and by pressure on the cover-glass we can determine that they lie embedded in a colourless mucilage. On the stalk, under the columella, is usually to be seen a small collar, a relic of the lime crust which was attached there. In the protoplasmic lining layer of stalks which are not too old, we can follow fine, usually *longitudinal, streaming of the protoplasm. The sacs of Mucor* are **multinuclear,** the nuclei very small, only distinguishable by suitable fixing and staining. The gonidia, also, are multinuclear.

If uninjured material be transferred to absolute alcohol, chromic acid, or picric acid, and afterwards stained, the lining plasmic layer of the mycelium, as of the aerial gonidiophores, can be seen to contain numerous small nuclei, scattered at irregular distances, and connected together by plasmic threads. They are also recognisable in the gonidangia, and, though with more difficulty, in the gonidia. The best treatment for Fungi is to fix with weak Flemming's solution, and stain with saffranin, *gentian-violet, or orange. In most cases the saffranin should act* for half an hour, the gentian-violet for fifteen minutes, the orange for only a very short time. The preparation should then be rapidly washed with alcohol and oil of cloves.

Slide Culture of Mucor.—Mucor Mucedo is a very suitable object wherewith to be introduced to the methods of fungus culture upon the object-slide, and we will therefore amplify here the methods already learned in connection with bacteria and yeast. We prepare, when needed, a suitable culture-fluid, by boiling horse-dung in water. The infusion is filtered clear, and then again boiled for a long time in order to sterilise it. The *object-slides and glass-utensils needed for use must likewise* be sterilised by passing through a gas or spirit flame, or by being laid for a short time in absolute alcohol and then in ether, which latter will rapidly evaporate after removal. It has also been commended to preserve the glass-utensils in 10 per cent.

hydrochloric acid, to remove them just before needed for use, and wash them in distilled water, which has been boiling for some hours. Glasses cleaned in this way allow the drop of culture-fluid to be well spread out, a point of no small advantage. These precautions are rendered necessary by the existence in the atmosphere of various spores which might infect the culture. It is necessary now to sow a single gonidium, and this is effected in the following way. A gonidangium from a pure culture is transferred with the forceps to a watch-glass filled with boiled water. In this the gonidia will soon become uniformly diffused. A drop of the fluid can then be taken out of the watch-glass by means of a needle which has been disinfected in a flame, and laid in an elongated streak upon an object-slide. This streak is then examined under the microscope. If it contains but one gonidium, it is in a fit state for use for the culture; but if it contains more than one, a part of it must be wiped off with a scrap of boiled rag. A drop of the culture fluid must then be laid on the gonidium, the slide then laid upon one of the zinc frames represented in Fig. 1, and this covered with a bell-jar, the edges of which are immersed in water. It is even better to add a few drops of the culture-fluid to the watch-glass of water containing the gonidia, and to leave them there for a few hours. The gonidia swell to a ten-fold size, becoming globular, and are much easier to see and count in the streak upon the object-slide. The swollen gonidium will show a large central vacuole (Fig. 113*, *B*).

Several **germ-tubes** will quickly be developed from the gonidium, will grow rapidly, and, in the course of a day, as can be readily seen by repeated examination under the microscope, will produce a much-branched mycelium (Fig. 113*, *C*). Successive grades of branches progressively diminish in thickness. The entire mycelium is devoid of partition walls, and is filled with dense, granular protoplasmic contents, in which are numerous vacuoles. When the mycelium has attained a definite size, further branching ceases; the protoplasm becomes more granular and darker, and begins to collect towards the middle of the mycelium. Here the gonidiophore rises out of the fluid as a thicker branch; and when this has attained a certain size, the end swells into a head (Fig. 113*, *C*), the bulk of the protoplasm of the mycelium moves towards this rudimentary gonidangium (Fig. 113*, *D*), and is replaced by cell-sap. The gonidangium is cut off by a partition wall, which bulges into it (*E*), and its

contents separate into individual portions, which constitute the gonidia (*F*). When the gonidangium is ripe, the gonidiophore rapidly elongates. In the mycelium, partition walls have already

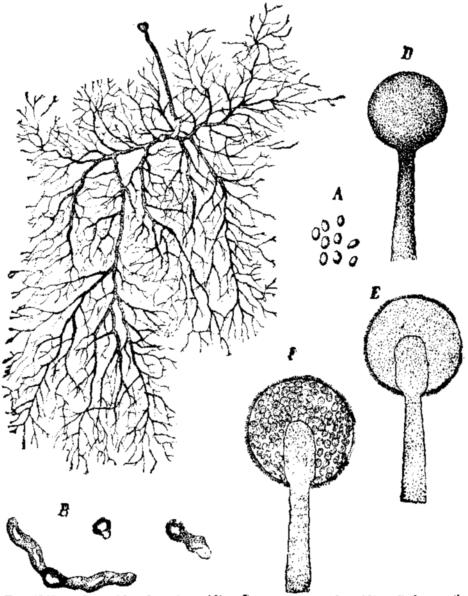


FIG. 113*.—*Mucor Mucedo.* *A*, gonidia; *B*, germination of gonidia; *C*, the greatly branched thallus (mycelium), proceeding from the gonidium, and commencing to produce erect gonidiophores, which are developed centrifugally; *D*, swollen end of a gonidiophore, still continuous throughout; *E*, separation of the gonidangium by a partition, so as to form the columella; *F*, formation of spores, separated by an interstitial substance; the wall of the gonidangium bristles with crystalline points of oxalate of lime. (From Van Tieghem's *Traité de Botanique*, after Brefeld.)

been formed. This stage of the development is attained in, at the outside, three days.

While we should by no means neglect to get an insight into

the rapid development of this fungus, it is well to state that, should we wish to carefully study the various stages as above, it will be necessary to start several similar cultures, since for observation a cover-glass must be laid on, and the preparation will thus be spoiled for further development. With a sufficiently high power, protoplasmic streaming will be readily recognisable, and especially along the wall of the gonidiophore.

Permanent preparations of spore-cultures, at any time prior to the elongation of the gonidiophore, can be made by fixing the object by carefully flooding the object-slide with one of the fixing fluids, and then staining the object while still upon the slide. In the centre of such a preparation the original gonidium can usually be still recognised as a slight swelling (*see* Fig. 113*, *C*).

Sexual Reproduction of Mucor[1].—On the object-slide we obtain only gonidiophores, ultimately several on each individual; in order to see the sexual organs and **zygospores (zygotes)**, we must make a culture *en masse*. They are most readily obtained upon horse-dung cultures, but rarely in quantity; so that we often look for them in vain. When present the zygospores show on the dung as black dots. If such a dot be carefully removed to an object-slide, we can, if it be actually a *Mucor* zygospore, recognise it as a black ball covered with wart-like projections. It is very readily torn, but if it should happen to be complete, we can recognise the two ends of darkly-coloured mycelial threads attached to it (Fig. 114, *C*). If the mycelial thread be torn off, or has already separated from the zygospore, the places of attachment can be seen as clear circular spots (Fig. 114, *D*). These can be seen with special ease when the zygospore is crushed. The contents of the zygospore consist, as is then seen, of finely granular protoplasm and oil. Besides the ripe zygospores, we may also find younger, paler, or even colourless ones which do not yet possess the warty prominences; and we may even have portions of mycelium in which the formation of zygospores has just commenced. We then see two mycelial threads, rich in contents, the ends of which have swollen spherically, which have joined together by their apical surfaces (Fig. 114, *A* and *B*). At a little distance from these surfaces each of the swollen ends has been cut off by a partition wall. In somewhat older stages, the contact surfaces of the two sexual organs have disappeared, and the contents of both cells have mingled. The **zygospore** (**zygote**), which has thus resulted from the copulation or

[1] See note on p. 325.

conjugation of two similar cells, rounds off and enlarges considerably, and the two attached, club-like, swollen mycelial threads form the **suspensors.**

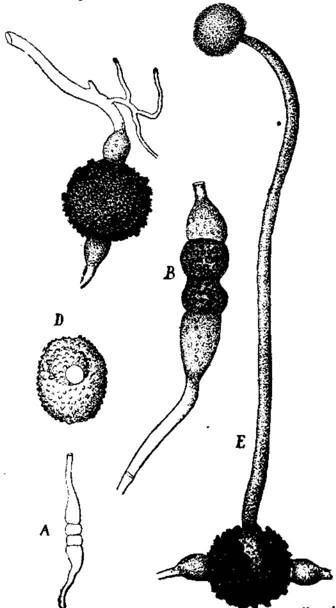


FIG. 114.—*A*, contact of two branches, and separation of two cells, which will form the zygospore; *B*, fusion of the cells, enlargement of the zygospore and of the branches which bear it; *C*, ripe zygospore, enclosed in the blackened membrane of the conjugated cells; *D*, zygospore isolated from this outer membrane and upon which we see one of the rings of attachment; *E*, zygospores germinating in moist air into a sporangiferous hypha. (From Van Tieghem's *Traité de Botanique*, after Brefeld.)

The formation of zygotes can usually be forced in the months of March and April, if gonidia are sown in fresh, flattened-out horse-dung. The zygotes are ready in from eight to fourteen

days. At other times, in order to obtain zygotes, it succeeds well if the sowing is made in some drops of concentrated plum-juice (*e.g.*, of French plums), sterilised by long boiling, and then mixed with 10 to 20 per cent. of absolute alcohol. The sowing is made on a cover-glass in a damp chamber constructed of a glass ring (see p. 279), and the object-slide placed in the large plaster of Paris moist chamber (p. 279).

That these zygospores actually belong to *Mucor Mucedo* can be determined by germinating them. When the conditions for their development are present, the zygospores are produced in great number, and a large amount of material for investigation can be obtained by cleansing the dung in question with water. The ripe zygospores sink. They are carefully washed and laid upon object-slides under a bell-jar with its edges immersed in water. Germination begins in about six weeks, when each zygospore emits usually one thick germ-tube, which is a **sporangiophore**, and is crowned by the characteristic sporangium of *Mucor* (Fig. 114, *E*). For the emission of the germ-tube the black outer wall of the zygote, the **exosporium**, is only torn so far as is necessary; the development of the sporangium proceeds relatively slowly, and is completed about the third day after the commencement of germination.

"*Mucor Yeast.*"—*Mucor* can be grown also upon the surface of a saccharine fluid, and forms submerged and aerial hyphæ as upon bread or dung. But if gonidia are grown *completely submerged* in such a fluid, as, for example, in the thin layer between a cover-glass and a slide, the hyphæ produced break up by constriction into necklace-like strings, the units of which separate and bud, much after the fashion of *Saccharomyces* (p. 288), and like it induce active alcoholic fermentation.

Parasites on Mucor.—In studying dung-cultures of *Mucor Mucedo*, it is well to note that it is commonly accompanied by one, or two, other Mucorineæ, *Chætocladium Jonesii*, and perhaps *Piptocephalis Freseniana*, which grow parasitically upon it. The mycelial threads of *Chætocladium* unite with the mycelial threads and gonidiophores of *Mucor*, by means of the resorption, at the place of union, of the separating walls. Numerous further prominences arise, and unite with the *Mucor* hypha as a suctorial apparatus, or **haustoria.** The mycelial threads of *Piptocephalis*, on the other hand, cling to the *Mucor* threads by swollen ends, from which numerous delicate processes penetrate into its interior.

If note is not made of these relations, the fructifications of these two parasitic fungi might be regarded as belonging to the *Mucor* itself.

Moist Chamber Cultures of Micro-Fungi.—In the cultivation of fungus spores in moist chambers it may be remarked that for rapid cultures the pasteboard chambers answer admirably; but that for cultures which last more than a few days they cannot be used, as they themselves form a nidus for the spores of various moulds. Glass chambers (p. 279) must then be used. The infusion of horse-dung recommended for *Mucor* does not keep for long, and therefore can be recommended only for cultures of rapidly-developing moulds. If the development requires a longer period, it is sometimes feasible every other day to remove the drop carefully with a pipette, and replace it with a new supply. The infusion keeps the longest when the dung is stirred in water, boiled and filtered, and the filtrate kept for at least twenty-four hours in a vapour bath. In many cases a cold extract of dried fruits, such as raisins, pears or plums, is very serviceable. Such an extract is filtered till clear, and then evaporated to the thickness of syrup. It can be kept for years unchanged, and when wanted for culture purposes can be mixed in suitable proportions with well-boiled water. If the fluid has an acid reaction it must under some circumstances be neutralised with ammonia, as many fungi cannot endure the acids derived from fruits.

The Potato Disease, Phytophthora infestans.—The cause of the potato disease is likewise a Phycomycete, *Phytophthora infestans*, de Bary, the germ-tubes of which penetrate through the membranes of the epidermal cells of the potato-leaf into its intercellular spaces, and, spreading about in these, destroy the tissue of the host-plant, forming brown spots of constantly-increasing diameter. In order to obtain the fungus in quantity in a fructifying state, we place a piece of a diseased potato-plant in an atmosphere saturated with moisture under a bell-jar, and let it lie there for about two days. The diseased leaves are now covered over on both sides, but especially on the under, with white "mould," formed by the filamentous fruiting branches (**gonidiophores**) of the *Phytophthora*, which are especially developed at the edges of the brown spots. On surface sections of the parts thus covered we see the gonidiophores projecting through the widely-opened **stomata**. We can demonstrate this,

though less completely, upon fragments of leaves, which we place bodily under the microscope. The gonidiophores appear as delicate, unseptate threads, branched above, and filled with finely-granular protoplasm (Fig. 115, *A*). The branching is monopodial (or racemose); the number of branches usually only two or three. These branches are irregularly swollen at intervals. In dry air the gonidiophores, collapsing, are twisted upon their axis. Here and there we see at the end of a branch a gonidangium in course of development; the ripe lemon-shaped gonidangia, however, have fallen off in laying the preparation in water. In order to find the gonidangia on the gonidiophore, we must examine the preparation dry, covering it with a cover-glass, and running a trace of water under it from the edge, as otherwise the gonidiophores, as already indicated, rapidly drying, shrivel up. In plants collected from the open air the gonidiophores are found only on the under side of the leaves, and do not grow so tall as in the moist chamber; are much less noticeable, therefore, with the naked eye.

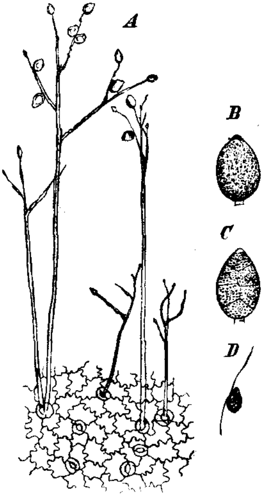


FIG. 115.—*A*, surface view of the epidermis of the leaf of *Solanum tuberosum* (the potato), with the gonidiophores of *Phytophthora infestans* projecting out of the stomata (× 90); *B*, a ripe gonidangium; *C*, another, with divided contents. *D*, a swarm-spore (*B-D* × 540).

Delicate *cross-sections* through the margin of a spot on a diseased leaf, made by means of elder-pith, permit us to clearly follow the exit of the gonidiophores from the stomata. Often several such hyphæ come side by side out of the same stoma; or, more commonly, the hypha branches at its exit, and gives rise to gonidiophores in proportion. From these places we can, though with difficulty, follow the hyphæ inwards, into the tissue

of the leaf, and determine that they pass into the **intercellular spaces.** From the mycelium arise numerous extraordinarily delicate filamentous **suctorial organs (haustoria)**, which penetrate *into the cells of the host-plant.* The delicate mycelial threads, on the other hand, cling closely to the cells of the host. These cells show first a browning of their chlorophyll grains; these ultimately fuse together, and with the other constituents of the cell-contents, into a dark-brown, coagulated mass; at the same time the whole cell collapses. The gonidangia are lemon-shaped (Fig. 115, *B*), with short stalks, somewhat tapering apex, and finely granular contents. The membrane is very delicate, a little swollen at the apex. As we have already seen, they are situated at the ends of the branches of the gonidiophore; if they have attained their full dimensions, the apex of the branch under the point of origin of the gonidangium proceeds to grow unilaterally, and presses the gonidangium over to one side, so that this comes to lie in a position at right angles with the branch. At the apex of the branch soon arises the rudiment of a new *gonidangium* (compare Fig. 115, *A*).

We sow *the gonidangia in a drop of water upon a cover-glass*, and take care by stirring the drop that the greater part of the gonidangia are immersed. The cover-glass is laid upon a small moist chamber, with the drop suspended. The culture must not be carried on in too strong light. After the lapse of about an hour, perhaps later, the formation of **swarm-spores (zoogonidia)** from the contents of the gonidangium begins; hence we have named *these structures gonidangia, not gonidia*; they can, however, germinate direct, functioning as gonidia, in which case we see some of those lying at the surface or at the edge of the drop put out a germ-tube from the anterior papilla. In those which are immersed and form swarm-spores, the contents divide into an indefinite number of cells (*C*), in each of which we can see a small central vacuole. The apex of the gonidangium swells out into a papilla, is finally dissolved, and the separated masses of its contents are successively pressed out through the small round aperture. They hasten away as swarm-spores (zoogonidia). If we fix these swarm-spores with iodine solution, we can determine the presence upon them of two cilia. These are inserted laterally, near the now peripheral vacuole (*D*). The movement of the swarm-spores lasts up to half an hour. They then come to rest, surround themselves with a cellulose membrane, and

soon produce a germ-tube. It is this tube, developed direct from the gonidangium, or from a swarm-spore, which penetrates through the epidermis into the stem and leaves of the potato-plant, and can in this way, as may be proved, infect a completely healthy plant. The rapid multiplication of the parasite is provided for by the formation of gonidangia and swarm-spores.

Sexual organs have not yet been found upon *Phytophthora infestans*, although known for the nearly allied Peronosporeæ. In these, mycelial branches in the interior of the host swell, usually at their end, spherically, and form the **oogonia** by cutting off these swellings by partition walls. By each oogonium is found a mycelial branch, with its end cut off as an **antheridium.** The greater part of the protoplasm present in the oogonium forms a central globular egg-cell or **oosphere.** The antheridium puts out a fertilising sac to the oosphere, and the resulting **oospore** surrounds itself afterwards with a firm membrane.

Pythium.—One of the best of the **Oomycetes** for study, and an exceedingly likely one in which to obtain the sexual organs, is *Pythium de Baryanum*, one of the group of parasitic thread fungi which are responsible for the disease known (from its results) as the "damping off" of seedlings. This disease, often disastrous in its widespread results, is specially induced in seedlings grown under unhealthy conditions, *e.g.*, too closely crowded, too wet, insufficiently exposed to light and air, etc. The particular fungus named above occurs almost with certainty upon seedlings of common garden cress, *Lepidium sativum*, when thickly sown in a flower-pot about two-thirds filled with soil or cocoa-nut fibre refuse, covered with a glass plate, set in a saucer of water, and kept super-plentifully supplied with moisture. The seeds germinate normally, but in a few days the hypocotyl will be seen to bend over, owing, apparently, to a serious spindling of the stem just above ground level. A few days later the lower parts of the stems will be seen to be enveloped in a feltwork of fungal hyphæ, which, commencing in each case near the base of the hypocotyl, spreads gradually over the entire plant, causing its decay. Over the surface of the ground the hyphæ may spread to, and infect, other plants.[1]

If now a portion of a collapsed stem be examined in water

[1] See an account, illustrated, by H. Marshall Ward, in *Q. J. M. S.*, vol. xxiii., p. 487 *et seq.* Also Trow, in *Ann. Bot.*, vol. 15, 1901. See note p. 325.

with a low power, the colourless hyphæ will be seen spreading over the surface of the stem, and especially crowded at the position of collapse, while the external tissues of the host will show manifest signs of disorganisation. Teasing out a fragment with needles, the hyphæ are seen to be highly refractive, abundantly branched, only here and there septate, spreading over the surface, and penetrating it, occasionally by the stomatic clefts, but generally by perforation through the epidermis. Within the host-plant the hyphæ can be traced, partly in the intercellular spaces, partly in the cortical cells themselves, the solution of the walls being effected by means of a **cellulose-ferment.**—If a healthy cress seedling be placed in a watch-glass of water for twenty to twenty-four hours, with a fragment of an infected seedling, the attack of the healthy seedling by hyphæ from the infected one can be followed, and in a few days **gonidia** will be developed. Many of these will be seen terminating branches, within or outside the host-plant, in the form of pear-shaped, and later on, spherical swellings; while many others will be found in intermediate parts of the hyphæ; so that the gonidia can be either **terminal** or **interstitial.** The gonidia are filled with granular protoplasm; each is cut off by a septum. They are **asexual** reproducing bodies. If the tissues are allowed simply to decay, these gonidia are set free, and lie dormant as **resting spores.** If, however, fresh water is added, or they are transferred to a fresh specimen, they germinate in a few hours, one or more germ-tubes being produced. Even in a fresh supply of water, with no *Lepidium* material, they will germinate, but quickly die. If sown immediately after separation the gonidia produce **zoospores,** resembling those in Fig. 115.

If a piece of *Lepidium* material, with a large number of gonidia, be placed in a pretty considerable quantity of fresh water the **sexual organs** can often be readily produced, even in a few hours; or a small well-selected piece may be cultivated in a suspended drop. Examination at frequent intervals is desirable, and the development of the oogonia and antheridia, and processes of fertilisation may be followed. With patience in observation the same structures may often be found developed from gonidia in the general culture.

In general appearance the **oogonium** resembles the terminal gonidium, is spherical, and cut off from the parent hypha by a septum. When fully formed the contents of the oogonium have separated into a central granular mass, the **oosphere,** and

a clearer enveloping layer, the **periplasm.** The **antheridium** is developed shortly after the oogonium in the form of a short club-shaped cell, cut off by a partition wall, and arising either from immediately below the oogonium, on the same hypha, or from some neighbouring hypha. This antheridium grows into close contact with the oogonium. In its contents also a segregation into a central fertile portion and a surrounding periplasm is recognisable. No spermatozoids are formed. The antheridium puts out a short cylindrical fertilising-tube, which penetrates into the oogonium, traverses the periplasm, and reaches the oosphere; its end opens, and the central contents of the antheridium traverse it, and coalesce with the oosphere, fertilising it. At a later stage the **oospore** may be found, surrounded by a thick cell-wall, in part at least derived from the periplasm, which has disappeared, so that the oospore lies loosely in the oogonium. The contents of the oospore are densely granular, and with many oil globules. It also is a **resting spore,** but of a much more resistant character than the resting stage of the gonidia.

Penicillium crustaceum, or Blue Mould.—Upon the most various objects in damp positions, even if the merest traces of nourishment can be obtained from them, soon is wont to be found the blue-green mould, *Penicillium crustaceum,* Fries. It is the most widely distributed of all moulds; we meet with it everywhere, and shall not, therefore, need to seek long for material for examination. It will be, however, most convenient to moisten a piece of bread, and place it under a bell-jar. Not improbably Mucorineæ will first show themselves on the bread; but soon the, at first, more slowly-developed *Penicillium* will have supplanted it, and after about a week covers the substratum with a dense blue-green covering. The blue-green colour arises from the gonidia of *Penicillium,* which, however, only show this colour when in great quantity.—We now lift a little material from the substratum, and examine it in water.[1] The **mycelium**

[1] In the examination of fresh objects in water, it often happens that air clings very tightly amongst the hyphæ, and hinders observation. Attempts to remove the air have usually disadvantageous effects upon the preparation itself. In order to render the gonidiophores of these and other moulds free of air, as far as they will admit of it, and yet having their natural distribution, the following method may be used: A scrap of the material is laid carefully upon the surface of a drop of glycerine, a drop of alcohol is placed upon it, and the cover-glass is laid on. The extraordinarily violent diffusion currents which are set up usually displace the air quite ely.

consists of branched hyphæ, divided by numerous partition walls. The contents directly visible are finely granular protoplasm and small vacuoles. Individual threads, not distinguishable from other mycelial threads, have developed into fruiting branches (**gonidiophores**). At their apex they produce a whorl of short branches, which branches (Fig. 116, *s'*) on their part either bear directly whorls of **sterigmata** (*bst*), or each one again bears a whorl of shorter branches, and then these bear the whorls of sterigmata. This branching gives to the gonidiophore the appearance of a brush. Commonly other lateral branches, which arise just under a partition wall of the primary gonidiophore (as in the right hand of the figure), come up laterally to this terminal brush, and form secondary gonidiophores. The sterigmata, as sufficiently strong magnification shows, are cylindrical, prolonged at their end to a finer projection (*st*). This swells spherically at its apex, and forms a quickly-growing gonidium. Under the first gonidium a second swelling soon appears, which becomes a gonidium, and so on; so that chains of gonidia arise, the terminal one being the oldest. The uppermost gonidia of the chain are thrown off, while new ones are thrust outwards from below.

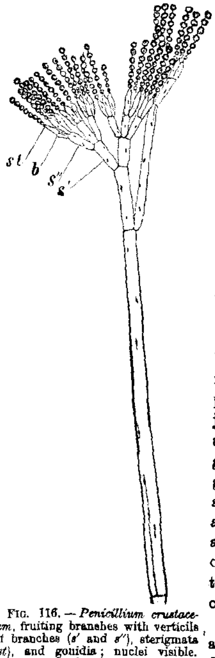


FIG. 116. — *Penicillium crustaceum*, fruiting branches with verticils of branches (*s'* and *s''*), sterigmata (*st*), and gonidia; nuclei visible. From an alcohol-hæmatoxylin preparation (× 540).

Tufts of *Penicillium*, fixed with alcohol, stain very well with very dilute hæmatoxylin, by which it can be determined that in the cells of the mycelium and the fruiting branch numerous **nuclei** are present. The nuclei are very small, so that they require strong magnification. They are elongated in the longitudinal direction of the cell, and joined together by fine plasma threads. In long

cells very many can be counted; in the shorter branches of the whorls on the gonidiophores, only one or two; in the sterigmata, probably only one at the upper end. The sterigmata, however, are usually filled so thickly with contents at the apex that the identification of the nucleus in them is impossible. In the gonidia also, with the strongest magnification, a nucleus can be distinguished with certainty for each.

We may add that besides the above-described gonidiophores, it is possible to rear upon *Penicillium* a second kind of fruiting body. These arise in suitably managed culture *en masse*, have the size of small pin-heads, and a yellowish colour. In their interior, after longer period of rest, asci are formed, each of which produces eight spores (ascospores). Therefore *Penicillium* must be set down as an Ascomycete, one representing the section of cleistocarpous Ascomycetes, with closed fructification. Out of the spores developed in the asci the brush-like gonidiophores have been again developed upon the object-slide.

NOTE ON THE SEXUALITY OF THE MUCORINEÆ (p. 3[illegible]).

The uncertainty in production of zygospores, and abundance when produced at all, which has hitherto been attributed to the effects of external conditions, has recently received explanation by the discovery, by A. F. Blakeslee, of the *dioicism* of the Mucorines. A plant grown from one spore (gonidium) does not produce zygospores; similarly with a patch from spores of common origin; but plants derived from spores of varied origins, grown collaterally, may show them along the line of contact. Those cultures, therefore, are most promising which are grown from spores obtained from varied sources. See a critique by V. H. Blackman in the *New Phytologist*, November, 1906, p. 215.

NOTE ON PYTHIUM (p. 321).

There appears to be difficulty in some localities in making sure of the occurrence of the parasitic P. de Baryanum, and Trow suggests the use of one or other of the saprophytic species instead; indeed he considers the latter to have the advantage also in ease of study, the freedom with which they produce sexual organs, and the comparative readiness with which pure cultures can be obtained. The method adopted is that of the text-book, very rotten material being examined for spores contained in it. These spores, when found, are cultivated in the "hanging drop" in a moist chamber, fragments of boiled cress seedlings being used as nutrient material; on a larger scale Petri dishes, with dead flies or cabbage leaf, in water, as pabulum; or glass-pot cultures on boiled potato. See Trow, "Observations on the Biology and Cytology of *Pythium ultimum*," *Ann. Bot.*, v. 15 (1901), pp. 269-312 and Pl. 15, 16; and Miyaki on "The Fertilisation of *Pythium de Baryanum*," same vol. pp. 653-667 and Pl. 36.

CHAPTER XXIV.

[illegible]PRODUCTION OF THE HIGHER FUNGI AND OF LICHENS.

PRINCIPAL MATERIALS USED.

[illegible]lla esculenta; fresh, dried, or in alcohol.
[illegible]lus furfuraceus, on horse- or cow-dung; fresh.
[illegible]um Berberidis, on leaves of Barberry in May and June; fresh, or in alcohol, or dried and soaked.
[illegible]a graminis, on grasses in summer and autumn.
[illegible] species; or *Agaricus campestris*; fresh, or in alcohol.
[illegible]chia ciliaris; fresh, dried, or in alcohol.

PRINCIPAL REAGENTS USED.

Po[illegible]-Iodine.

Rep[illegible]ction of an Ascomycete, Morchella esculenta.—In order to inve[illegible] the structure of the hymenium of a highly-developed form of [illegible]comycetes, we can take the Morell, *Morchella esculenta*. Eve[illegible] specimens can here, after softening, be employed for th[illegible]igation. Fresh are naturally to be preferred. The well-kno[illegible] Morell has an irregularly ovate, stalked fructification, w[illegible]its interior conceals a simple cavity, and whose upper swo[illegible] has deep infoldings. The hollows or chambers between th[illegible] are clothed with hymenial tissue, while this is not deve[illegible] upon the projecting exposed ridges themselves. Suitable sec[illegible] are very easy to obtain, and must be taken perpendicularly to the surface of a hollow. The hymenium consists of approximately parallel spore-sacs (**asci**) and **paraphyses** (Fig. 117). The asci (*a*) are almost cylindrical, and contain in their upper part eight elliptic, unicellular spores, **ascospores**, crowded together. Besides the spores, there is also present in the asci the, in part, strongly refractive **epiplasm**. The paraphyses (*p*) are brownish, septate threads, somewhat swollen above. The uppermost cell (*p*) is especially elongated

They do not attain the length of the asci. Asci and paraphyses arise as free hyphal ends from the densely interwoven, flatly extended, sub-hymenial tissue. This arises from the more loosely-constructed inner hyphal texture of the fructification. Addition of iodine colours the masses of epiplasm in the asci reddish-brown. This reaction is characteristic of epiplasm, and is considered to be a reaction for **glycogen**. The characteristic peculiarity of this reaction is shown upon warming. To the preparation lying in water, and coloured by iodine, some water is added, yet not so much as to decolorise it; then it is carefully warmed, without attaining the boiling-point, and held over white paper, in order to see if the colour has become paler. When this happens, the preparation is rapidly cooled, and the darker coloration, in large preparations even visible with the naked eye, again appears. With the aid of iodine, the base of many asci can be traced pretty deeply into the sub-hymenial tissue. The contents of the spores, paraphyses, sub-hymenial tissue, and of the tissue in the interior of the fructification colours at the same time yellow or yellowish-brown.

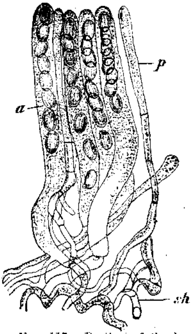


FIG. 117.—Portion of the hymenium of *Morchella esculenta*. *a*, asci; *p*, paraphyses; *sh*, sub-hymenial tissue (× 240).

Ascobolus furfuraceus.—When fresh horse-, or, still better, cow-dung is placed in a shallow glass vessel, covered with a glass lid, and allowed to stand in a moderately strongly-lighted place, a luxuriant fungus vegetation begins at once to develop. First appears *Mucor Mucedo*, with its parasites, as described on p. 317 later (upon cow-dung) comes still another Mucorine, which, by reason of its hemispherical black sporangia, thrown off by a special swelling layer, may be referred to *Pilobolus crystallinus*. After two or three weeks, when the Mucorines have for the most part completed their development, will appear the hymenomycetous *Coprinus*, with small cap and long stalk; at the same time may also appear on the substratum small pear-shaped cups, which are the fructifications of an Ascomycete of the section **Discomycetes**, belonging to the genus *Ascobolus*, and probably *A. furfuraceus*.

Dung-cultures so rarely fail to produce some of the Ascobolidæ that they may be considered the readiest means of obtaining material for the study of the Discomycetous Ascomycetes.

The small cups have a diameter of from $\frac{1}{25}$ to $\frac{1}{8}$ inch; their somewhat projecting base appears, according to circumstances, *dark brown or yellow. This colour often changes while under* observation—especially when the culture is examined in early afternoon—and, by lifting the cover, a sudden change is induced *in the state of moisture of the material and its surrounding atmo*sphere. It can then be determined that the dark masses, which are spores, have beeu flung out of the sporangia. If the vessel is not too high, microscopical examination of its cover will easily show the *Ascobolus* spores clinging to it, singly or in little heaps. The black heads of *Pilobolus* may similarly be clinging to the glass cover.

If we remove from the substratum a fructification of *Ascobolus*, a portion of the mycelium upon which it arises will probably accompany it. It is a brownish feltwork, formed of thick hyphæ, septate by cross-walls. We prepare between finger and thumb, or in other fashion, delicate *longitudinal sections* of the fructification, and examine it under the microscope. The foot, tapering downwards, is formed of pseudo-parenchymatous tissue. The segments of the hyphæ are considerably swollen, rounded or polygonal by lateral pressure, of relatively considerable dimensions, but very poor in contents. Upwards, the cells of this foot decrease in size, but increase in coutents, up to the very dense, richly protoplasmic **sub-hymenial tissue.** From the broadly *expanded sub-hymenial tissue arises the* **hymenium,** *formed of* **asci** and **paraphyses.** The foot and hymenium alike are surrounded by a **cortex,** composed of globular, somewhat firm-walled, elements. These elements, in the main arranged in but a single layer, are not clearly delimited from the inner tissue of the foot. The asci contain eight spores, **ascospores,** the walls of which are at first colourless, then become violet to brown. Simultaneously with this change of colour a beautiful tracery becomes visible, brought about by a delicate striation of the cell-wall. Quite characteristic is the colourless, usually unilateral, swelling, a gelatinous appendage, which each spore possesses, and which is especially visible upon the spores while still colourless. Study of their development shows that here also the entire protoplasm of the ascus is not used in forming the spores, and it is easy to find

in the preparation young asci which, besides the spores, show also a protoplasmic residue—the **epiplasm**. During the ripening of the spores this dwindles to a thin lining layer. The tip of the ripe ascus projects above the surface of the hymenium. On its flattened apex a small circular cover is clearly recognisable. This is opened for the discharge of the spores, the expulsion of which is brought about by a strong tension upon the walls of the ascus, due to a strongly swelling substance surrounding the spores. It is this substance, also, which so greatly stretches the ascus that its apex protrudes from the hymenium; the empty collapsed asci are withdrawn into the hymenium.[1]

The **paraphyses** are long, much septate threads. Their terminal segments are swollen club-wise; their contents are sparse and colourless. Asci and paraphyses are in their upper part embedded in a gelatinous substance of a sulphur yellow colour, and it is this which gives to the entire fructification its yellow hue. This likewise proceeds from the swollen membranes.

On the addition of normal iodine solution the hymenium, in its lower parts, takes on a blue colour,—a phenomenon which rarely comes with the Fungi, but is widely spread amongst Lichens. These latter, in point of fact, are also, like *Ascobolus*, almost always Ascomycetes, but living symbiotically with Algæ. The red-brown staining of the epiplasm in the asci appears here less beautifully than in *Morchella*. According to the colour of their walls the spores take, at the same time, a light brown to a dark brown colour.—By the aid of their gelatinous appendage the spores remain clinging to the substratum against which they happen to have been propelled. The spores do not, however, germinate, but, as researches have shown, must pass into the intestines of an animal in order to become capable of germination.

The formation of the fructification of the Ascomycetes results from a **sexual act**.[2] As a first rudiment, an oogonium and an antheridium are present. After the union of the oonucleus with the male spermonucleus, the oogonium grows to a thick multicellular thread, a cell of which swells, and, in *Sphærotheca*, develops direct into the ascus, of which but one is present in a fructification; in *Ascobolus*, on the contrary, a branched thread arises from it, which finally develops the asci. The young asci are mostly multinuclear; in *Sphærotheca* two, in *Ascobolus* four nuclei. These original nuclei, however, combine with one another

[1] See note on p. 338.

[2] This has been proved in a few Genera only.—[ED.]

into the ascus-nucleus, which then, by successive bipartitions, gives rise to the eight spore-nuclei.

Puccinia graminis.—In the months of May and June may perhaps be found upon the under side of the leaves of the Barberry[1] (*Berberis vulgaris*) orange-coloured warts, which, to the naked eye, appear finely pitted. Examination with a lens shows them as cushion-like yellow swellings, upon which are placed small orange-red cuplets. The corresponding positions on the upper side of the leaf appear as reddish spots edged with yellow. Examined with a lens, usually numerous brown dots, surrounded with orange-red, show in the inner parts of them. Individual similar dots are often to be found on the edges of the cushion on the under side of the leaf. The cuplets on the cushion of the under side of the leaf are the **æcidium-fruits** of *Æcidium Berberidis*, the "cluster-cup" of the Barberry; the corresponding dots on the spots on the upper side of the leaf, and also upon the edges of the cushion on the under side of the leaf, are the **spermogones** appertaining to them. Together they form the first generation of the common fungus, Rust of wheat, etc. (*Puccinia graminis*), belonging to the **Æcidiomycetes** or **Uredineæ**, of which the second generation is passed through upon our corn and other grasses, giving rise to the appearance of the disease called "rust".

By means of elder-pith we prepare delicate *cross-sections* through an infected leaf, and examine them with a low, and afterwards with a high power. We assume that fresh material stands at our disposal; the investigation can, however, be carried on satisfactorily upon dried and soaked, and very well upon alcohol material. The sections prepared from the fresh leaf are especially clear if we run in a little potash solution. In the uninfected parts the barberry leaf shows, proceeding from above downwards: an **upper epidermis**; a single layer of elongated **palisade-cells**; a loose **spongy parenchyma**, about five cells deep; the **under epidermis**. The cushions of tissue of the infected parts have attained more than double the thickness of the leaf. Upon the palisade layer of the upper side, which is higher, but otherwise appears little changed, impinges a closed

[1] This plant is now rare in many parts of Britain in the wild state, having been exterminated by farmers for reasons which the life history of *Puccinia* will render obvious. The commonest Æcidiomycete is probably that which forms the rounded yellow swollen patches on the leaves of the Coltsfoot.—[ED.] On the Uredineæ, see V. H. Blackman, in *Ann. Bot.*, v. 18 (1904), 323-73 and Pl. 21-24.

tissue, more or less elongated in a direction at right-angles to the surface of the leaf, and, from the small development of its **intercellular spaces**, is essentially distinguished from the spongy parenchyma of the surrounding parts of the leaf. The epidermis of both surfaces of the leaf has not been affected in form. The contents of all these cells are disorganised, and consist partly of colourless oil drops, partly of greenish-yellow and reddish drops and granular masses, proceeding from the chlorophyll grains and the cytoplasm. The entire tissue of the cushion shows its *intercellular spaces traversed by delicate, septate, slightly-branched* fungal **hyphæ**, containing oil drops. These extend on both sides to the epidermis. With chlorzinc iodine, as also with iodine and sulphuric acid, blue coloration is not induced, since **fungal-cellulose** rarely shows this reaction. The **æcidium-cups**, as we have them before us in longitudinal section, are sunk more than half in the tissue of the cushion. We easily determine that the mycelial hyphæ under the cups form a dense, almost pseudo-parenchymatous, layer, from which, perpendicularly outwards, and parallel to one another, rise numerous thicker club-shaped hyphæ, without interspaces, forming the so-called **hymenium.** These hyphæ, the **basidia,** pass over at their ends into straight rows of **spores,** which at the basidia are colourless, and, from mutual pressure, polygonal, but gradually become orange-red and rounded. Higher up the spores separate from one another, and are evacuated from the opened cup. The observation of the youngest spores upon the basidia convinces us, however, that they are cut off one after another by cross-walls, from the apex of the growing basidia. The unilamellar wall of the fruit (the **peridium**) consists of cells which look very like the spores, but remain polygonal, and do not separate laterally from one another. Their fine delicately porous walls are especially strongly thickened on the outer side. The developing peridium pushes back and destroys the surrounding tissue of the cushion, and tears open the epidermis in order to open out to the exterior.

The pear-shaped **spermogones,** especially found upon the upper side of the leaf, are, like the æcidium fruits, surrounded though less strongly, by a weft of hyphæ, from which arise densely-crowded parallel threads, running towards the middle line of the structure. These threads are very delicate; those found in the upper part of the organ project as delicate bundles towards the exterior (compare Fig. 118 later). These delicate threads, the

sterigmata, abstrict at their points exceedingly small, globular cells, the **spermatia**, which are evacuated from the organ in a mass of slime. The sterigmata themselves contain orange-red oil drops, which give to the entire organ, especially in its outer parts, its special colour. The spermatia germinate slightly in culture fluids, but not, apparently, in nature. The spermogones correspond morphologically with the similarly named structures in the lichen-forming Ascomycetes, and are doubtless male sexual organs which have become functionless.

As already mentioned, the fungus lives as a second generation upon **Gramineæ**. It belongs to the **heterœcious** parasites, which, in contradistinction to the **autœcious**, go through their **alternation of generation** upon different hosts. This has been demonstrated by direct sowing of the æcidium spores upon seedlings of the cereals. The **uredo** condition of *Puccinia graminis* we encounter not infrequently in corn fields, from mid-June to autumn, upon rye, wheat, barley, oats, and especially on the couch-grass or twitch (*Triticum repens*). It is found chiefly on the haulm and the leaf-sheaths of the infected plants, and is easily recognised as narrow, rust-coloured to dark-brown streaks, running parallel with the veins. Upon the leaf-sheaths and haulm (stem) these streaks attain to even two or more inches in length. The epidermis of the host is torn open and raised by the protruding gonidial tissue. First appear the rust-coloured patches of the summer spores, the **uredo-spores**, with which are gradually associated brown winter spores, **teleutospores**. These usurp the place of the uredospores, and at length completely supplant them, whereon the patch becomes dark brown, almost black. Towards the end of summer only teleutospores are to be found.

If fresh material is not obtainable, that preserved in alcohol, and even dry plants, softened in water, will serve for study. We first prepare a *cross-section* through the haulm of an oat which is infected with rusty uredo-streaks. We can easily demonstrate upon the cross-section that the fungal hyphæ only traverse definite tissues of the host, *viz.*, the chlorophyll-containing, looser strips of tissue, which alternate with sclerenchymatous strips in the periphery of the stem, and are covered with an epidermis provided with stomata. Here the cells are densely enveloped in hyphæ, and their contents disorganised. In the places where the section has passed through a uredo-streak, we can see numerous short and delicate branches, directed

outwards, arise from the mycelium, which from their swollen ends abstrict a unicellular spore, the **uredospore.** The surface is ruptured, its edges thrown up laterally. The spores are in different stages of development. Those that are ripe appear an elongated oval, and with sufficiently strong magnification we can distinguish two layers in their wall. The outer, dark brown, is covered with numerous small warts; the inner, less dark, shows several, usually four, regularly-distributed pits in the equator. The contents of the spore are granular, in the interior parts a lively orange-red.

Cross-sections through the haulm of oat, bearing the dark-brown streaks of **teleutospores,** show the same structure, as far as the hyphæ are concerned, as we have already seen. The teleutospores are mounted upon stalks similar to those of the uredospores, but thicker walled. The teleutospores are two-celled. The two cells together form an obovate body, somewhat tapering at its two ends, and with a dark-brown wall. Plants examined in the course of the summer may have uredo- and teleutospores simultaneously in the streak.

We may add that these teleutospores hibernate, and are, therefore, of the nature of **resting-spores**; and are first capable of further development in the next spring. Each of the two cells puts out a delicate germ-tube, the so-called **promycelium,** which at its upper end cuts off four cells; each of these puts forth a short awl-shaped outgrowth, which cuts off at its apex a kidney-shaped **sporidium.** These sporidia can only infect, *i.e.*, germinate upon, the Barberry leaf; if they happen upon a sufficiently young leaf, their germ-tube pierces straight through the outer wall of the epidermal cell directly into the interior of the host. As we therefore see, the way through the stomata, by which the germ-tubes of the æcidio- and uredospores enter, is not the only one by which infection is possible.

The uredo-stage of *Puccinia graminis* can hibernate upon winter corn and propagate the rust next summer, with omission of the teleuto- and æcidium stages.

Reproduction of a Hymenomycete, Russula.—In order to study the structure of the **hymenium** of the **Hymenomycetes,** it is preferable to use one of the numerous species of Toadstools (*Amanita*), Mushrooms (*Agaricus-Psalliota*), or *Russula*. We will select a *Russula*, because this possesses **cystidia** also. The cap or **pileus** shows on the under side radially-arranged lamellæ

or gills. These bear the hymenium. We cut, parallel to the course of the lamellæ, a small piece out of the cap (pileus), and make through this *cross-sections* perpendicular to the course of the lamellæ; these must be as thin as it is possible to make them. The entire cross-section appears like a comb, on which the

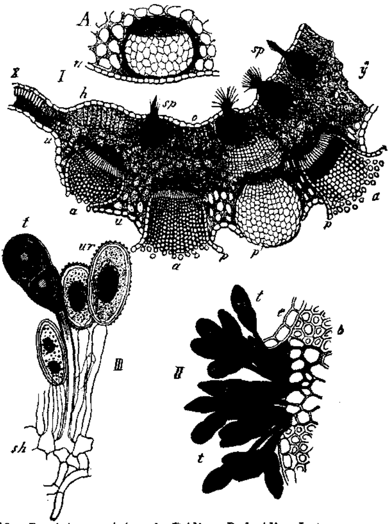


FIG. 118.—*Puccinia graminis* and *Æcidium Berberidis*. I., transverse section of the leaf of *Berberis*, with æcidia (*a*); *p*, the peridium, or wall of the æcidia; *u*, under, *o*, upper epidermis of the leaf; from *u* to *y''* the leaf has become thickened by the action of the parasite, thus forming the cushion; on the upper surface are spermogonia (*sp*). *A*, a young æcidium which has not yet burst. II., layer of teleutospores (*t*) on the leaf of *Triticum repens*; *e*, its epidermis. III., part of a layer of uredospores on the same plant; *ur*, the uredospores; *t*, a teleutospore. (After Sachs.)

sections through the lamellæ form the teeth (see Fig. 120, *A*, later on). With a low power we see that the hyphæ pass out of the cap into the lamellæ, run rectilinearly in the middle of these, and continuously give off ramifying branches, which are directed obliquely towards the flanks of the lamellæ, and again branch

(see Fig. 120, *B* and *C*). Some of these branches swell into club form, and end blind; a larger proportion remain slender, and form, at the base of the club-shaped swollen branches, a dense layer of short, rounded segments, which we will distinguish as the **sub-hymenial layer.** This is limited more or less sharply towards the inner tissue of the lamella — the **trama.** The club-shaped swollen branches of the trama serve to give to the lamella the necessary stiffness. From the sub-hymenial tissue spring the **basidia,** and **paraphyses** (Fig. 119). These have an approximately parallel course, stand out perpendicularly from the sides of the lamellæ, and form the **hymenium.** The basidia (*b*) are club-shaped. On their flattened ends they form four, symmetrically placed, thin branches—the **sterigmata** (*s*). These swell gradually at their apices, each into an elliptic spore—**basidiospore** (*sp*). The basidiospores, when they have attained their full dimensions, remain in most cases smooth, or, in many species of *Russula* (*cf.* Fig. 119), they form short spines on their surface. They are then cut off from the sterigma by a partition wall, and ultimately fall off[1]. The septation and separation takes place a little below the spore-swelling, in the position where the sterigma shows a slight nick. The fallen spore has therefore a short stalk. Between the basidia stand the shorter sterile **paraphyses** (*p*).

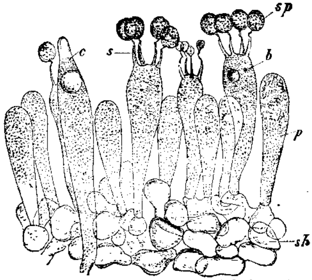


FIG. 119.—*Russula rubra.* Part of the hymenium. *sh*, sub-hymenial layer; *b*, basidia; *s*, sterigmata; *sp*, spores; *p*, paraphyses; *c*, a cystidium (× 540).

So far the Toadstools and Mushrooms agree with the above-described *Russula.* In *Russula*, however, between basidia and paraphyses occur also isolated **cystidia** (*c*), structures of the size of the basidia, which project a little with their pointed ends above the hymenial surface, and with their tapering base penetrate to the sub-hymenial tissue, showing themselves to be direct branches of the median elements of the trama. All the elements in ques-

[1] They are probably discharged explosively.—[ED.]

tion are cut off at their base by partition walls; they contain finely granular protoplasm, and not infrequently isolated oil-drops.

The accompanying Fig. 120 will further elucidate the structure of the Hymenomycetes. It is taken from the common Mushroom, *Agaricus campestris*. There are no cystids, and each basidium produces only two, instead of four, basidiospores. The Mushroom offers, moreover, the advantage of being obtainable fresh all the year round. *Coprinus*, already referred to as coming freely upon dung-cultures, makes, while young, admirable material for the study of a simple hymenomycetous fungus, entire longitudinal sections being taken through the unexpanded pileus.

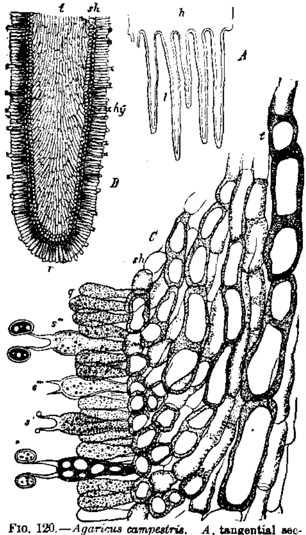


FIG. 120.—*Agaricus campestris*. *A*, tangential section of the pileus (*h*), showing the lamellæ, *l*. *B*, a similar section of a lamella more highly magnified; *hy*, the hymenium; *t*, the central tissue, or trama; *sh*, the sub-hymenial layer. *C*, a portion of the same section still more highly magnified (× 550); *g*, young basidia and paraphyses. At this stage these are practically alike; *s′*, first formation of conidia on a basidium; *s″*, *s‴*, more advanced stages; at *s⁗*, the conidia have fallen off. (After Sachs.)

Reproduction of Lichens, Anaptychia.—The fungi taking part in the formation of the thallus of Lichens belong, with few exceptions, to the Ascomycetes. *Anaptychia ciliaris*, already known to us, fructifies very freely. The apothecia are saucer-shaped, on a support developed from the thallus. This contracts under the apothecium like a stalk. A cross-section through this stalk shows radial structure, with a fairly uniformly thick cortical layer, and a homogeneous gonidial (Algal) layer. The interior of the stalk

is occupied by a **medulla**, or "pith," formed of a looser hyphal texture. We prepare *median longitudinal sections* through the apothecium. This shows us the apothecial support formed of the tissue of the thallus. The gonidial layer extends to its rim, which grows out in places into ciliary outgrowths. The apothecial support has expanded into a bowl-like form, in order to admit the **hymenium**, which arises from the medullary tissue. The hymenium is recognisable by its somewhat brownish colour. It consists of very numerous, long, exceedingly narrow, septate threads—the **paraphyses**; between these, but far less numerous, stand the club-shaped sacs—the **asci**. These latter are always of various ages; the ripe ones contain eight brown-walled spores—**ascospores**. These spores are elliptic, two-celled, slightly constricted at the boundary of the two cells. Paraphyses and asci arise from a like-coloured, felted, horizontally expanded layer of little thickness, which is distinguished as the **sub-hymenial layer**.

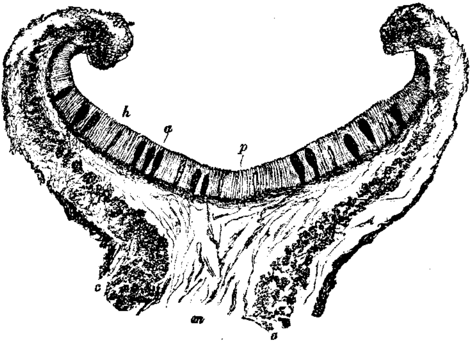


FIG. 120*.—An apothecium from *Anaptychia ciliaris*; *h*, hymenium; *a*, asci; *p*, paraphyses; *c*, cortical layer; *m*, medulla, or pith; *g*, algæ or gonidial layer (× 45).

This originates from the medullary tissue of the stalk, from which it is delimited by its brownish colour and the want of air-containing spaces. While, as we have seen, the hyphæ of the thallus itself are not coloured blue even by chlorzinc iodine (p. 240), those of the hymenial tissue take a dark-blue colour, even with

the addition of a little iodine. The walls of the hymenial elements are formed of a special modification of cellulose, which has been distinguished as **starch-cellulose.**

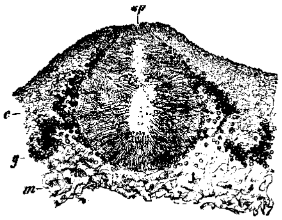


FIG. 121.—Cross-section through the thallus of *Anaptychia ciliaris*.

If we examine the thallus of *Anaptychia ciliaris* with the lens, we shall notice here and there upon it wart-shaped prominences, standing singly or in groups. If in such places delicate cross-sections are taken in considerable numbers, we shall probably cut through such a swelling (Fig. 121). It appears then as an ovate structure, sunk in the thallus, and opening outwardly with a pore, and is now known as a **spermogonia.** It occupies almost the entire depth of the thallus, is laterally surrounded by the algal layer, and in the interior shows itself to be constructed of delicate, shortly segmented, approximately radially-arranged threads, single or in bundles (compare the Figure). The long axis of the organ is traversed by a cylindrical cavity, which receives the rod-like **spermatia,** which are segmented off from the ends of the threads. Through the upper opening of the spermogonium the spermatia can make their exit. They are probably the male sexual organs of the Lichen; but, so far as capable of separate germination, are also *conidia*.

If *Anaptychia* is not at our disposal, various other lichens show pycnidia, such as *Parmelia (Physcia) parietina, Verrucaria nitida, Collema melænum,* or *Cladonia rangiferina.*

NOTE ON THE EXPLOSIVE DEHISCENCE OF ASCI (p. 329).

An exceptionally beautiful illustration of the explosive discharge of ascospores is provided by the large cup-fungus *Peziza aurantiaca*, not infrequently found in gritty turf in damp shady places. If the hymenial surface of the fungus is gently stroked with the finger, asci will dehisce in great numbers, and the spores be discharged in clouds, quite visible to the naked eye; resembling, indeed, the pollen clouds of the well-known "Artillery Plant". In still air the distance to which the spores are propelled is several inches, while they are carried further with the smallest aerial movements. With care, and under favourable conditions, the spore clouds can be shown upon a screen.—[ED.]

CHAPTER XXV.

THE REPRODUCTION OF MOSSES AND LIVERWORTS (BRYOPHYTA).

PRINCIPAL MATERIALS USED.

Marchantia polymorpha (Liverwort), with gemmæ; preferably fresh.

The same, with male and female receptacles. Preferably fresh. May be kept in alcohol.

Male "flowers" of a Moss, *e.g.*, *Mnium hornum*, or *Polytrichum*. Fresh, dried, or in alcohol. (*Mnium hornum* is very common in woods and on shady banks.)

Female "flowers" of the same. Fresh, dried, or in alcohol.

(Both of these gathered in April, May or June.)

Spore-capsules of the same. Fresh or in alcohol.

PRINCIPAL REAGENTS USED.

Osmic acid, 1 per cent.—Iodine.

Vegetative Reproduction of Marchantia.—The universally distributed Liverwort, *Marchantia polymorpha*, the structure of which we studied in chap. xviii., rapidly multiplies vegetatively by its **gemmæ**. These are common amongst liverworts in general, and are here met with in especially beautiful form. The gemmæ of *Marchantia* arise upon the dorsal (upper) surface of the thallus in cup-shaped receptacles or **cupules** (see Fig. 122, *B*). The cups have a beautifully-toothed rim, and at their bottom the bright green gemmæ are visible.

A *median longitudinal section* through the cupule, parallel to the long axis of the shoot which bears it, shows that the cup is at first slightly narrowed upwards, and then somewhat suddenly expands into the broad rim. The tissue which forms the air-chambers passes into the exterior of the cup, to above the point where its outward broadening begins. The bottom of the cup is occupied by unicellular club-shaped **papillæ**, the membrane of

which swells into mucus. Between these papillæ are also found some which are two-celled; and some also, the upper cell of which has been further cross-septate. The lower cell remains simple, and forms the **pedicel** (or stalk); the cells of the upper portion soon again divide longitudinally, and the structure becomes constantly more multicellular, enlarges much tangentially, and becomes several cells thick in the middle. Others will be found which have attained their ultimate biscuit-like or bi-convex form as fully-developed **gemmæ**. The unicellular pedicel can easily be broken through. The separation of the gemmæ, and their ejection from the cups, results from the strongly-swelling mucus, which is developed from the unicellular club-like papillæ at the bottom of the cup. Each of the two lateral indentations of

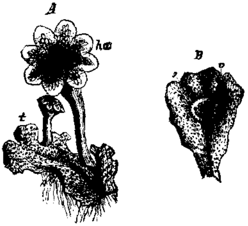


FIG. 122.—*A*, a portion of a thallus of *Marchantia polymorpha* (*t*), with the upright male receptacle (*hu*), bearing antheridia. *B*, portion of a thallus with a cupule containing gemmæ; *vv*, growing points of the two branches of the thallus. (After Sachs.)

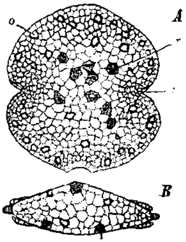


FIG. 122*.—Gemma of *Marchantia polymorpha*, *A*, in surface view; *B*, in section; *v*, growing point; *o*, oil-cells; *r*, rhizoidal cells (× 75).

the gemma conceals a growing point, protected by short papillæ. The cells of the gemma are rich in chlorophyll, but on both sides large colourless cells occur, in the median portions only, but otherwise irregularly scattered. At the edge, individual cells contain oil-bodies. After the dissemination of the gemmæ, the large colourless cells develop in one or two days into hair-roots (**rhizoids**), in all cases on the shaded side of the gemma only, which hence becomes the ventral side, while the side exposed to the light forms morphologically the upper or dorsal side. If the gemma be inverted the new under side similarly becomes ventral, and produces rhizoids.

Sexual Reproduction of Marchantia.—The sexual organs of the Marchantiaceæ are situated upon special receptacles, which we will also study in *Marchantia polymorpha*. **Male and female**

receptacles are readily distinguishable; the former shield-shaped, with scolloped outline (Fig. 122, *A*), the latter radiating like bare umbrella ribs. The two sexes are situated upon different plants, *i.e.*, the plants are diœcious; the receptacles and their stalks are metamorphosed branches of the thallus.

We prepare between elder-pith delicate *longitudinal sections* through the male receptacle, and can determine that its upper side has exactly the same structure as the dorsal surface of the thallus, and that similarly the under side resembles the ventral surface of the thallus, and bears rhizoids and scales. On the *upper* side, however, sunk in special cavities, are the antheridia (Fig. 123, *A*). On suitable sections we can see that in each cavity is found only one antheridium, besides some short unicellular paraphyses (*p*); the cavity contracts above the antheridium into a narrow canal. The antheridium is a shortly-stalked, oval body, with a unilamellar chlorophyll-containing wall. The mother-cells of the spermatozoids (antherozoids) have been produced by successive divisions at right angles, and even in the almost ripe antheridium still form cross and longitudinal rows (see Figure). Each of these cells finally divides yet once more, and gives rise to two spermatozoids. Shortly before the ripening of the antheridium, the mother-cells round off, and become disconnected, the antheridium ruptures at its apex, and they are evacuated. If a drop of water is placed upon a fully-developed receptacle, the water is seen rapidly to spread over its whole surface, and soon becomes milky. If this water is now examined with a high power, we can see in it innumerable evacuated spermatozoidal cells. They remain at rest only a short time, when the cell-membrane swells; finally it is ruptured, and the spermatozoid escapes into the surrounding water. The spermatozoids are

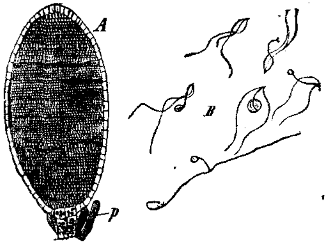


FIG. 123.—*Marchantia polymorpha.* *A*, an almost ripe antheridium in optical cross-section; *p*, paraphyses. *B*, spermatozoids (antherozoids), fixed with 1 per cent. osmic acid ($A \times 90$, $B \times 600$).

comparatively minute, have a thread-like body and two long cilia; to the posterior end clings a vesicle, which is lost during the swarming. In order to see them clearly, we run into the preparation a drop of 1 per cent. osmic acid, and as they are fixed beautifully by the reagent, we can now study them conveniently (Fig. 123, *B*), or we can use for the same purpose a *trace of iodine solution*. The cilia can be seen with especial clearness in dry preparations, which are obtained by allowing a fresh preparation, or one which has been fixed in any way, to dry slowly and perfectly without covering it with a cover-glass. Such preparations can then be covered with a cover-glass, and can be closed in any suitable way and preserved.

The **female receptacle** forms, like the male, a radially-spreading inflorescence; and in general there are nine rays, and between them are eight rows of **archegonia** on the under side of the receptacle. The distinction from the male receptacles is striking, in that here the sexual organs stand upon the under side; but this phenomenon is connected with an early displacement of the growing point towards the under side of the receptacle. Under the simple microscope we can demonstrate that each row of archegonia, lying between two rays, is enclosed in a common veil-like covering, fringed at its edges.—We prepare, between thumb and forefinger, delicate *longitudinal sections* through a comparatively young receptacle, and upon some of these sections find, without difficulty, the female sexual organs, the **archegonia.** The oldest lie nearest the edge, the younger progressively nearer the stalk. The first ripening archegonia show along the edge of the disk with their neck curved upwards, the succeeding ones hang straight down. In an approximately ripe archegonium (Fig. 124, *A*) we can distinguish a short stalk or **foot**, a ventral portion, the **venter**, and a **neck.** The wall of the venter, as of the stalk, is unilamellar. The **central cell** of the venter is filled by the **oosphere**, and the **ventral canal-cell** (k''), which is cut off from it shortly before ripening. In the oosphere the nucleus is readily visible. The neck is traversed by the **neck-canal**, which is composed of a series of four **neck canal-cells**, the walls between which are dissolved, and the disorganised contents thus fused into a connected string. Between the archegonia arise numerous small leaf-like scales of the receptacle. In many preparations we can see the unilamellar veil-like covering, fringed

at its edges, which protects the entire row of archegonia. Numerous cells of this contain oil-bodies.

Fertilisation.—It is comparatively easy to see the opening of the archegonium take place under the microscope. We take quickly *longitudinal sections* through a female inflorescence, which has not yet raised itself, or only a little, upon its stalk, lay it dry under a cover-glass, and examine it under the microscope. When we appear to have found a ripe archegonium, and while still keeping it in view, we place a drop of water at the edge of the cover-glass. After the entrance of this, the archegonium opens almost immediately. The cause of the opening lies in the strong swelling of the contents of the neck-canal. The neck-cells separate from one another at the apex of the neck. The contents of the neck canal-cells pass out, then the contents of the ventral canal-cell follow. The homogeneous portion of these contents is formed of a strongly-swelling slime, which diffuses in the surrounding water; the granular contents remain in the surrounding water, where they are slowly disorganised. Immediately after the ejection of the ventral canal-cell, the oosphere in the central part of the venter rounds off (Fig. 124, *B*). At its anterior margin, *i.e.*, that in apposition to the canal, a clearer spot, the receptive-spot, is often, though not always, to be distinguished.

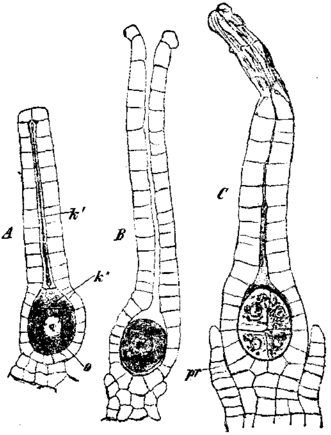


FIG. 124.—*Marchantia polymorpha.* *A*, young; *B*, opened archegonium; *C*, fertilised archegonium after the commencement of the formation of the embryo. *k'*, neck canal-cells; *k''*, ventral canal-cell; *o*, oosphere; *pr*, perianthium (× 540).

The penetration of the spermatozoids into the neck-canal can also be easily *observed*. *For this purpose, instead of pure water*, we add to the preparation a drop which has previously lain on a male receptacle. The spermatozoids quickly collect in the slime expelled from an archegonium; we see them enter the neck, where they become invisible. A substance (which in the closely-allied mosses is cane-sugar) is given off from the archegonium, which acts as a chemical stimulus, and determines their direction *of movement*. Thus *they get into the slime given off from the* archegonium, in which they slowly move in the direction of the opening of the neck.—It is interesting to prove that in an unfertilised archegonium the neck does not close, and under such conditions the archegonium slowly decomposes. If, on the other hand, water containing spermatozoids is added to the preparation, and the oosphere becomes fertilised, the neck closes, even after a few hours, contracting progressively from above downwards. Keep the preparation, and after twenty-four hours the presence of a cellulose membrane around the fertilised oospore is easy to recognise. In the course of the next few days the thickness of this cellulose wall increases.

Structure of the Sporogonium.—The fertilised archegonia, which we may find upon the *longitudinal sections*, show a shrivelled and brown neck, while the *oospore* has divided (Fig. 124, *C*). Around the base of the archegonium, from its foot, a cup-shaped sheath, the so-called **perianthium (perigynium)** (*pr*) begins to develop. This soon encloses the entire swollen archegonium. — Upon longitudinal sections of receptacles which have already spread out their radiating ribs, we see the bright green, swollen archegonia, with base broadened to correspond, situated upon the surface of the receptacle, and crowned by the remnant of the neck. —From the oospore gradually proceeds the **sporogonium**, which we see in *longitudinal sections*, prepared from still older receptacles. The sporogone consists of a shortly-stalked, oval, yellowish-green capsule. The wall of this capsule is unilamellar; if we spread it out with needles, and examine it with stronger magnification, the characteristic thickening rings in the otherwise thin-walled cells will appear. The yellow-walled **spores** are finely punctate. Between them lie narrow, long cells, tapering at both ends, and distinguished each by two brown spiral bands on its wall; these are the **elaters.** The interior of the capsule is entirely filled with spores and elaters. In capsules already opened (dehisced), we

can see that dehiscence takes place at the apex by means of a number of recurved teeth. The elaters are strongly hygroscopic, bend to and fro with hygroscopic changes in the atmosphere, and so assist the dissemination of the spores.

The sexual organs are not raised upon special receptacles in all the Marchantiaceæ, and in other Liverworts this structure is altogether wanting. On the other hand, the stalk of the sporogonium in many cases elongates considerably, and carries up the capsule with the spores, thus assisting the dissemination of the spores.

Antheridia of Mnium.—The **antheridia** of the leaf-bearing **Mosses** are best examined in a genus which has striking male "flowers". We choose a representative of the genus *Mnium*, *e.g.*, the widely distributed *Mnium hornum*, which in May and June "flowers" very freely, and bears female flowers and sporogonia at the same time. The male flowers are, it is true, much more striking than the female, and it is often necessary to search longer for these latter. The male flowers are dark-green, disk-shaped, surrounded by a rosette of leaves, the **involucre** or **perichætium.** Towards the interior of the flower these leaves decrease rapidly in size. In the axils of the outer, but chiefly, however, of the inner, perichætial leaves, stand numerous **antheridia** and **paraphyses**, which also spread over the entire apex of the axis. This is easily shown by *median longitudinal sections* of the flower, which are best prepared between the fingers, turning the apex downwards in cutting. On these longitudinal sections we see that the flower-axis broadens, after the fashion of a floral receptacle, at the place of insertion of the sexual organs, and in the middle is even a little hollowed. The central conducting bundle, peculiar to species of *Mnium* (see p. 228), has undergone a corresponding broadening, and ends in a chlorophyll-containing tissue, which spreads out under the receptacle. The antheridia and the paraphyses are at once recognised as such, and their structure is easy to understand (see Fig. 125 *A*). The **antheridia** are club-shaped, shortly-stalked bodies, somewhat tapering at both ends. The cells of their wall contain numerous chlorophyll grains. Where the longitudinal section has opened an antheridium, we see that its wall is composed of a single layer of cells. The contents of the antheridium consist of small, colourless cells, the partition walls of which in young stages of development clearly show rectangular arrangement. The ex-

truded contents of older antheridia opened by the section prove to be composed of rounded cells, still adhering together, the **mother-cells of the spermatozoids,** in which the thread-like body of the spermatozoid is already often recognisable. The chlorophyll grains at the apex of ripening antheridia assume a somewhat brownish tone. Emptied antheridia are open at their apex. The **paraphyses** are simple cell-rows, the cells of which gradually enlarge upwards, when they are, however, at least the uppermost again tapering; hence the uppermost cell is always pointed. The walls of the cells are often browned in the lower part of, and not infrequently even higher up upon, the paraphyses; they contain chlorophyll. *Cross-sections* through the lower part of the flower show in an instructive manner the distribution of the antheridia, their relations with the perichætial leaves and the paraphyses, and also provide us with numerous cross-sections through the antheridia.

Male Flowers of Polytrichum.—Still more striking than the male flowers of *Mnium* are the red-coloured ones of various species of *Polytrichum*, likewise found in May and June. For examination we choose *Polytrichum juniperinum*. The outer leaves forming the perichætium, beyond their colour, further differ from the ordinary leaves in that their unilamellar sheathing portion is continued up to the apex of the leaf. The green *lamellæ*,[1] characteristic of the genus *Polytrichum*, are found only towards the end or apical portion of the leaf, and almost always confined to the midrib. On the rapidly-decreasing reddish-brown perichætial leaves, near the interior of the flower, the green lamellæ are developed only on the extreme, sharply outward-bent points. The leaf thus appears ultimately reduced almost to its sheath portion alone. The antheridia and paraphyses stand in the axils of the perichætial leaves. The middle of the flower is, however, occupied by a vegetative bud, into which the central bundle of the stem is continued. Thence arises the later growth through the male flowers **(proliferation),** which is normal for *Polytrichum*. The antheridia have the same structure as in

[1] The leaves of *Polytrichum*, though really unilamellar, like those of other mosses, are rendered opaque by being more or less covered by vertical green scales, or lamellæ, produced upon their upper side. In *P. juniperinum* each foliage leaf shows about forty-eight such lamellæ, running, as usual, longitudinally, and from four to six cells long.—[ED.]

Mnium. The paraphyses, forming in their lower part a long cell-row, usually broaden at their tip into a spathulate unilamellar cell-surface.—If a male flower of *Polytrichum* is squeezed slightly between the fingers, the contents of the antheridia come out as a milky slime, clearly visible against the reddish ground.

The form of the antheridium of Mosses varies very little, and the accompanying Fig. 125 of that of a moss especially common upon shaded cinder-paths and other places where the substratum has been burnt, upon walls in green-houses, etc., *viz.*, *Funaria hygrometrica*, will serve to illustrate it.

FIG. 125. — ***Funaria hygrometrica.*** *A*, an antheridium bursting; the body of the antheridium shows its wall of cells containing chlorophyll grains; *a*, the antherozoids (spermatozoids) (× 350). *B*, the spermatozoids more strongly magnified; *b*, in the mother-cell; *c*, free antherozoid of *Polytrichum* (× 800). (After Sachs.)

Archegonia of Mnium. — The female flowers of *Mnium hornum*, however, are not nearly so recognisable as the male, and it is often necessary to seek for them longer. The plants bearing them are far shorter than the male, and somewhat darker in foliage. The upper leaves close together, after the fashion of a bud, in order to protect the female sexual organs, the **archegonia.** As is shown by *median longitudinal sections*, the apex of the flowering axis is not broadened to any extent, but greatly blunted, and from this we can judge when we are dealing with a female flower, even if we do not happen at once to find the archegonia. The central conducting bundle of the stem is somewhat swollen under the receptacle, and ends, just as under the male flowers, in a chlorophyll-containing tissue. The modified leaves which form the female **perichætium,** while remaining leaf-like, decrease in size towards the middle of the flower; the apex of the flower is occupied by only a few archegonia, so that it is necessary to take strictly median sections in order to disclose them. The archegonia are constructed essentially like those of the Liverworts (see Fig. 126), but the foot is far more strongly developed, only tapering a little downwards, and forms the greater part of the lower half of the archegonium, and hence the **oosphere** appears comparatively small. We must look for it close under the com-

mencement of the neck, which here appears only a little narrower than the ventral portion. The chlorophyll contents of the cells make the archegonium anything but transparent, and hence the oosphere and the canal-cells of the neck usually need addition of potash to make them visible. In the axils of the perichætial

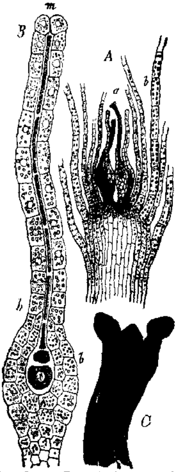


FIG. 126.—*Funaria hygrometrica*. *A*, longitudinal section of the summit of a weak female plant (× 100); *a*, archegonia; *b*, leaves. *B*, an archegonium (× 550), ventral portion with the oosphere; *h*, neck; *m*, mouth still closed; the cells of the axial row are beginning to be converted into mucilage. *C*, the part near the mouth of the neck of a fertilised archegonium, with dark-red cell-walls. (After Sachs.)

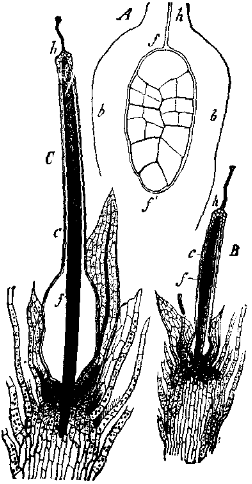


FIG. 127.—*A*, origin of the sporogonium (*f f'*) in the ventral portion, (*b b*) of the archegonium, seen in longitudinal section (× 500). *B*, *C*, different further stages of development of the sporogonium (*f*), and of the calyptra (*c*); *h*, neck of the archegonium (× about 40). (After Sachs.)

leaves stand numerous **paraphyses.** Each consists of a row of short cells, swelling somewhat upwards. The lowermost cells of these paraphyses have often become brown.

Sporogonium of Mnium.—**Fertilisation** in the Mosses takes place in all essentials as in the Liverworts, already described.

The **sporogonium**, the so-called "moss-fruit," the study of which we will carry on upon the same *Mnium hornum*, consists of stalk or **seta**, and **capsule.** The base of the seta is sunk in the tissue of the mother-plant. The result of fertilisation is here, therefore, somewhat different to that in the Liverworts, and needs a few words of explanation, further illustrated by Fig. 127. After fertilisation, the **oospore** develops into an embryo, an early stage of which is shown in Fig. 127, *A*. This embryo develops in length both upwards and downwards; downwards it grows into a foot which, as the base of the seta, passes through the tissue of the foot or stalk of the archegonium, and plunges into that of the apex of the moss-stem. (See Fig. 127, *B* and *C*). Upwardly, the embryo develops into the capsule, to be hereafter described. The seta remains for a long time short. Accompanying the increase in length, and likewise in thickness, of the young sporogonium, the body of the archegonium, which had enclosed the oosphere, also undergoes further development, keeping pace with the sporogonium in its growth, so as continuously to cover it. The upper part of the neck shrivels, as shown in Fig. 127, *B* and *C*, at the top. When, later in the development of the sporogonium, the seta rapidly elongates, the body of the archegonium, etc., is ruptured round its base, and is carried upwards, covering the capsule as with a cap, the **calyptra.** This calyptra, proceeding from the enlarged archegonium, which covers the growing capsule, is in *Mnium* early cast off, so that it is usually difficult to find. It is split up one side to its tapering apex, and is composed of one, in part also two, layers of elongated cells. The narrowed apex ends in a brown point, which indicates the remains of the neck of the archegonium. At the base, where it was ruptured by the growing sporogone, it appears as if cut off. The apex of the capsule, denuded of its calyptra, has a cover or lid (**operculum**) provided with a short beak. With a needle it can be easily loosed, when the edge of the capsular urn, fringed with its teeth, comes to view. The teeth form the **peristome.** The upper part of the seta, passing into the capsule, is called the **apophysis.** In the present case this last is separated from the capsule by a very slight constriction, and is distinguished from it by its brown colour. In some mosses, the apophysis is far wider than the capsule.

The Peristome.—In order to study the structure of the peristome, we take a *cross-section* through the capsule, close under

the brim of the urn, lift it up, and place it, with the teeth turned upwards, upon an object-slide. We remove the mirror from the microscope, or turn the diaphragm so that no aperture lies under, and observe the object with direct light. For this we can use only a low power. We can decide that the teeth are inserted in the inner brim, that they are wedge-shaped, and cross-striate. If we breathe lightly on the object while still looking at it, we shall see the teeth curve together inwards. They are hygroscopic; in damp weather they bend inwards, and so close the open capsule, while in dry weather they bend outwards, and again open the capsule. We count sixteen teeth on the urn. — We now lay the same section in a drop of water, and, tearing it through on one side with the needles, spread it out flat, cover it with a cover-glass, and observe it by transmitted light, and first from its outer side. We then notice, quite at the edge of the urn, a double layer of obliquely-arranged cells, papillately prolonged, pretty strongly thickened, and containing abundant chlorophyll grains. These cells have colourless walls, browned only at their very base, and there they are very easily disconnected from the edge of the urn, remaining, however, connected together. By means of these cells the separation of the operculum (lid) is effected; they form the so-called annulus at the rim of the capsule. Now laying it with the inner side upwards, the preparation shows us that the cross-striæ already noticed on the teeth are ridges projecting from their inner surface. Besides the outer peristome formed by the teeth, an inner one is also present; it consists of the so-called cilia. *Mnium hornum* has, therefore, a double peristome, while there are Mosses with only one, and also without any such peristome. The cilia, like the teeth, are here flat lamellæ, which in their lower part appear divided into chambers, and in their upper part cross-striate, by slight projecting ridges on their inner surface. In the lower part they are fused together into a continuous membrane, which bulges somewhat between each pair of teeth of the outer peristome. Two cilia stand between each pair of teeth, and present themselves obliquely from the rim. Their edges, the outer in their entire height, the inner only in the upper part, are fringed with small serrate projections. In these the cross-ridges of the surface of the cilia end. Through these serrations the pair of cilia in their upper part are combined by the outer edge, and finally the two fuse into a single narrow, elongated apex. With these pairs of cilia alternate very small ones, which, from

three to five in number, stand in front of the teeth of the outer peristome.

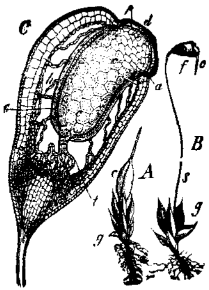
A delicate *cross-section*, taken somewhat deeper through the capsule, shows in the centre of this a column formed of large-celled tissue, the **columella.** Around this columella lies the cavity filled with spores. The inner wall of this is formed by the columella itself, the outer by a layer of tissue, usually two cells thick, and containing chlorophyll, which appears separated from the wall of the spore-capsule by a very loose chlorophyll-containing tissue. The **wall** of the capsule consists of two or three layers, and is covered by a sharply-defined **epidermis.** The cells of this latter are more strongly thickened on their outer walls. The **spores** contain chlorophyll grains, their wall is brownish, and studded with tiny warts; in favourable cases a three-sided pyramidal tapering of one side of the spore can be noticed, which arises from the tetrahedral position of the quartette of spores inside their mother-cell; it indicates the contact surfaces of its three sister-spores.

A perfectly *median longitudinal section*, which we prepare from a capsule which is still green and provided with its operculum, but is already fully formed, shows us uppermost the lid, consisting externally of one sheath of browner, strongly-thickened cells, and internally of many layers of thin-walled cells. At the limits between lid and urn lies the double layer of the obliquely arranged, chlorophyll-containing cells, already known to us, by which the separation of the lid is effected. The adjoiuing brown cells of the urn helow are distinguished by their small height. Similar cells adjoin these small ones towards the interior, and form thus an inward projecting ledge of thickened, brown cells, on which are set the teeth of the outer peristome. About the thickness of a cell removed arise the cilia. As the history of their development teaches us, these teeth and cilia arise by local thickening of the opposite walls of one and the same layer of cells adjoining the inside of the lid. The teeth proceed from definite portions of the outer walls, connected in the ascending direction; their cross-ridges indicate inner adjoining cross-walls, upon which the thickening has continued for some little distance. The cilia proceed from the thickened parts of the inner walls of this same layer of cells, and bear slight ridges at the places of junction of the next inner partition walls. In our median longitudinal section the lid is hollow; the inner tissue, after the formation

of the teeth and cilia, has shrivelled up, separating from the inner surface of the cilia, which extend to the top of the lid. This tissue now forms on the columella only a projecting conical knob. The **columella** is visible in its entire length; similarly we can survey the **spore-sac**, its outer wall, the looser tissue lying between this and the wall of the capsule, and lastly this wall. The spore-sac, so long as the lid has not been cast off, is closed above by a thin layer of tissue. Later on, it opens by the rupture of this layer. At the base of the capsule, under the spore-sac, an annular cavity has been formed. The **apophysis**, as is now seen, is provided with **stomata**, for on well-nigh every median longitudinal section, such will be cut. They lie below *the level of the epidermis; a pit leads down to each; an air-*chamber adjoins it internally. It is surrounded by chlorophyll-containing tissue, the intercellular spaces of which communicate with the annular cavity under the spore-sac, and with the intercellular spaces of the entire chlorophyll-containing tissue separating the wall of the capsule from the spore-sac. We have here, in short, an assimilating system, which enables the sporogone in part, if not altogether, to effect its own nutrition. — All the stomata in this longitudinal section are cut in the direction of their length, and give figures which, so far as can here be determined, agree with those of the Vascular Cryptogams, and of Phanerogams. This latter is so much the more striking since the apophysis (or, here and there, the wall of the capsule as well) is the only place in mosses where true stomata, constructed after the type of the higher plants, are borne.

In order to complete the impression we have obtained, let us now examine *surface-sections* of the capsule and of the apophysis. We can decide that on the surface of the capsule, stomata are wanting; between the brown-walled cells of the apophysis we see, however, pits which lead to the stomata. If we turn the section over, and examine it from the inner side, we can in favourable cases distinguish the two **guard-cells** of the stomata, formed as in higher plants. Upon such sections we can at the same time determine that the green cells, between the wall of the capsule and the spore-sac, are joined together in longitudinal series, that they are branched, and have all the aspect of algal threads. On cross-sections through the apophysis stomata have usually been cut, the two guard-cells of which are no difficult to see. At the seta, the differentiation of the epidermis

ceases; its surface is occupied by two or three layers of yellow to reddish-brown strongly-thickened cells, the cavities of which, as we pass inwards, become gradually larger. In the interior of the seta a central conducting bundle is differentiated. The stomata upon the apophysis of *Funaria hygrometrica* are of interest in that the division of the mother-cell, so as to form the guard-cells, is incomplete. Hence there is but one guard-cell, shaped like a ring, with a short median cleft. In the early



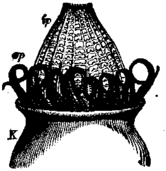


FIG. 129.—Mouth of the capsule of *Fontinalis antipyretica*, the operculum having fallen off. *ap*, outer peristome of teeth; *ip*, inner peristome of cilia (× 54). (After Sachs.)

FIG. 128. — ***Funaria hygrometrica***. *A*, a young leaf-bearing plant (*g*), with the calyptra (*c*). *B*, a plant (*g*) with nearly ripe sporogonium; *s*, its seta; *f*, the capsule; *c*, the calyptra. *C*, longitudinal section of the capsule bisecting it symmetrically; *d*, the operculum; *a*, the annulus; *p*, the peristome; *c*, *c*, the columella; *h*, the air-cavity (which here extends around as well as below the spore-sac); *s*, the spore-layer, consisting of the primary mother-cells of the spores (× about 20). (After Sachs.)

stages of development the dividing wall was complete, but its ends are subsequently resorbed.

The accompanying Figures 128 and 129 will serve further to elucidate the foregoing description of the structure of the capsule in mosses. It should be noted that the descriptions are confined to Bryaceæ, just as in the Liverworts they were restricted to the Marchantiaceæ.

NOTE ON FUNARIA HYGROMETRICA.

L. A. Boodle states that this moss is monoecious, the female branch being usually produced low down upon the very short male "plant"; and he is supported by A. H. Burtt as the result of examination of many thousands of plants sent out by the British Botanical Association, York. This is in agreement with the description of Bruch and Schimper ("Bryologia europæa"), but in opposition to the usual text-book statement (see *Ann. Bot.*, v. 20, p. 293-99, July, 1906).

CHAPTER XXVI.

THE REPRODUCTION OF THE PTERIDOPHYTA.

MATERIALS USED.

Fertile fronds of *Scolopendrium vulgare*, the Hart's-tongue fern. Fresh. (Alcohol material in part answers.)
The same of the Male Fern (*Aspidium Filix-mas*).
Fresh spores of *Ceratopteris thalictroides*, or *Osmunda regalis*, *Gymnogramme*, etc., for growing prothallia on peat, cocoanut fibre, tiles, etc.
Prothallia of *Polypodium vulgare*, or other fern. Fresh.
Fructifications of *Equisetum limosum*, or other species. Fresh and ripe; also unripe, fixed in alcohol, and softened in glycerine-alcohol.
Fructifying plant of *Selaginella Martensii*. Fresh, or dried. If dried material, soften with potash.

REAGENTS USED.

Gum arabic, 10 per cent.—Iodine—Chlorzinc iodine—Potash.

THE **sporangia** of Ferns stand, with few exceptions, on the under side of the leaves. They usually form groups, known as **sori**. The whole sorus is commonly covered by an outgrowth of the leaf—the **indusium**. The indusium can be very variously developed. If the edge of the leaf turns over the sorus, we speak of it as a **false indusium**.

Sporangia of Scolopendrium.—As an example for investigation we select the common Hart's-tongue fern, *Scolopendrium vulgare*. The leaf is traversed by a strong midrib, from which arise, usually at right angles, weak lateral veins. The sori are formed chiefly in the upper half of the fertile leaf. They have the same direction as the lateral veins. Externally they appear more or less completely covered by two, at first overlapping, lip-like indusia, which later on are more widely separated and spread open. It is only necessary to prepare a delicate *cross-section* of a piece of a fertile leaf, selecting a leaf on which the sori are already brown, but the edges of the indusium have not yet spread open. We cut with

the scissors a narrow strip out of the leaf, parallel with the sorus, clamp this strip between pieces of elder-pith (or pack several such strips together, one behind the other, in which case no elder-pith is needed), and take delicate cross-sections through them. The cross-section (Fig. 130, *A*) shows us an **epidermis** on the upper and under side, and a **spongy parenchyma**, the cells of which lie more densely together under the upper epidermis. The

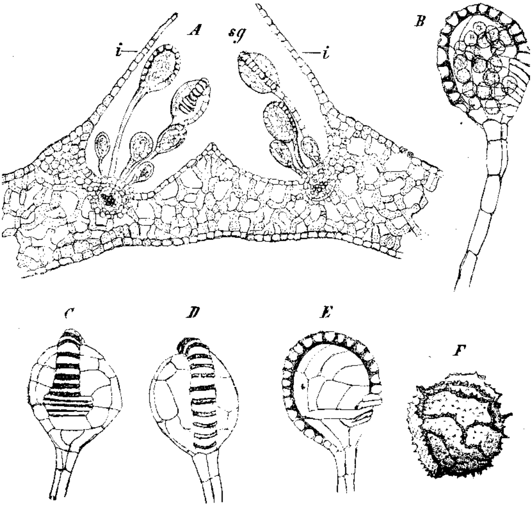


FIG. 130.—*Scolopendrium vulgare*. *A*, cross-section through the fertile part of the leaf; *i*, indusium; *sg*, sporangium, under each group of sporangia is seen the section of the stele. *B-E*, sporangia; *B* and *E*, seen from the flanks; *D*, from the dorsal side; *C*, from the ventral side; *F*, a spore. (*A*, × 50; *B-E*, × 145; *F*, × 540).

apparently simple linear sorus now appears divided into two. These stand right and left, inclined to one another, each in the angle between the leaf-surface and an indusium, and each close over a small stele. The surface of the leaf is here hollowed into a furrow, and between the two sori rises into a ridge. The epi-

dermis passes over into the **indusium** (*i*), which has the structure of the neighbouring epidermis, except that it is wanting in stomata and chlorophyll grains. In place of the latter it has small colourless chromatophores. From the base of the furrow arise the **sporangia** (*sg*); they can be seen in different stages of development; each derives its origin from a single epidermal cell. Even with a low power (Fig. 130, *A*) we can distinguish in each sporangium a **stalk** and a **capsule**, and on older ones a yellow-brown ring—the **annulus**—can be noticed on the capsule.—For further study we use somewhat stronger magnification (Fig. 130, *B*). The stalk passes over from a single to a double row of cells. The capsule has an unilamellar wall of cells. As is shown by different views of the wall of the capsule (*B-E*) the annulus is composed of a row of projecting cells of this capsule wall, which, commencing at the stalk, passes over the apex, and down the opposite side, and. flattening and becoming broader, dies away before again reaching the stalk. The inner and tranverse walls of the cells of the annulus are strongly thickened and browned; the thickening decreases in the transverse walls in the direction of the outer surface, and is wanting in the outer walls. The sporangium opens between the broad cells in which the annulus ends (Fig. 130, *C*, *E*); the one half of these broad cells then lies on the one, the other half on the opposite side of the fissure. The cause of the rupture lies in the annulus, which in drying tends to diminish its curvature. The brown wall of the ripe **spore** shows (*Fig. F*) its outer surface covered with a network of cockscomb-like projections.

Sorus of Aspidium.—In *Aspidium Filix-mas*, the "male Fern," we find indusia, kidney-shaped or sub-cordate, which with age become leaden-coloured, and finally brownish, shrivel somewhat, and no longer completely cover the dark-brown sori. The sporangia have almost the same structure as those of *Scolopendrium*. Upon some of them we see a short **glandular hair**, ending in an unicellular head, arise from the stalk. The sporangia are attached to a cushion-like prominence—a **placenta**—which lies over a stele. To this latter adjoin reticulately-thickened tracheïdes, which are distributed in the placenta. At its apex the placenta bears the indusium, attached by being curved down into the form of a stalk.

Dehiscence of the Sporangia.—If we take a preparation in water which includes sporangia that are ripe, but still unopened, and run in from the edge of the cover-glass a water-withdrawing

medium, best glycerine, the sporangia slowly open before our eyes. This is best done with *Aspidium; Scolopendrium* does not serve so well. The annulus thus becomes ultimately strongly concave. Then follows, with a jerk, an opposite movement, which more or less completely closes the sporangium. The entire phenomenon can, in lessened degree, be repeated once or several times. Careful observation shows that during the dehiscence the outer walls of the annulus project strongly into their respective cells.

This is due to the progressive loss of water by the cells of the annulus; so that the limbs of the U-shaped thickenings of these cells approximate to one another elastically. At length the cohesion of the water in the cells can no longer withstand the tension thus set up; rupture of the water takes place and at this moment commences the backward movement of the annulus. If this does not take place in all the cells, then in those in which it does not, the outward flexure still continues, thus bringing about the secondary movements of dehiscence. If now water is added to the glycerine, more water enters the cells and the sporangium almost completely closes. By renewed addition of glycerine the converse phenomenon can be once more brought about. If the sporangia open in the air, as is the case in the normal process of dehiscence, at the moment when the cohesion of the water in the annulus cells is overcome, air enters. The jerking dehiscence of the sporangia results in the spores being flung out to distances which, bearing in mind the size of the bodies concerned, are quite considerable.

The Prothallium.—We select the Ferns, likewise, for the purpose of studying the structure of the sexual organs in the group of Vascular Cryptogams, and of following the processes of fertilisation. The **prothallium**, which is the first and sexual generation of Ferns, is always easy to procure. To find prothallia in the open air is attended with considerable difficulty, and we shall therefore do well to look for them in plant-houses, where on damp shaded walls, on the stems of tree-ferns, or on flower-pots, we can almost always find them. On the fibrous peat, much used now in the culture of Orchids, *Sarracenia*, etc., and which is often permeated by *Polypodium vulgare*, are usually found numerous prothallia of this fern, which we will here select for closer study. As in most other Polypodiaceæ, the prothallia of the common Polypody fern have the form of small, heart-shaped, bright-green leaves, lying on the substratum. We seize a prothallium of medium size with the forceps, taking hold of the place where it is attached to the substratum, and lift it away. We immerse it in water, in which we move it for some time to and fro, in order to wash off the fragments of adhering soil, and then lay it, with the ventral (under) side upwards, in a drop of water on the object-slide, and examine it under a cover-glass. The prothallium, as already noted, is heart-shaped. It consists

of polygonal cells, containing numerous chlorophyll bodies. In the anterior indentation lies the small-celled **meristem** of the **growing point.** Only in its central portion is the prothallium multilamellar, as can readily be proved by changing the focus. This median portion is the so-called **cushion.** It passes over at the sides into the unilamellar portion, and slopes gradually also towards the base of the prothallium. From the hinder parts of the prothallium, *i.e.*, those farthest from the growing apex, arise the **rhizoids**; they are especially produced in the median line of the prothallium. They are long unicellular sacs, which soon become brown. At the edge and under side of the prothallium, individual cells, moreover, grow out into short, almost invariably unicellular, papillæ, which, like the rhizoids, are cut off by a partition wall at their base.

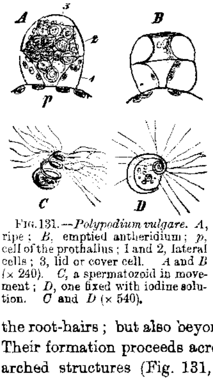


FIG. 131.—*Polypodium vulgare*. *A*, ripe; *B*, emptied antheridium; *p*, cell of the prothallus; 1 and 2, lateral cells; 3, lid or cover cell. *A* and *B* (× 240). *C*, a spermatozoid in movement; *D*, one fixed with iodine solution. *C* and *D* (× 540).

The Antheridia.—If we have chosen for examination comparatively young prothallia, they are male; if we have taken those which are too old, they bear exclusively female sexual organs. Intermediate are such as bear both sexual organs. The sexual organs, like the root-hairs, stand only on the ventral side of the prothallium. The male sexual organs, **Antheridia**, are found on the hinder parts of the prothallium; they arise between the root-hairs; but also beyond these on the flanks of the cushion. Their formation proceeds acropetally. They appear as spherical arched structures (Fig. 131, *A*), which, in a ripe condition, contain smaller, globular cells in great number inside a unilamellar wall. Farther behind the ripe antheridia stand those which are already emptied, recognisable by the browning of their inner walls, and a stellate gap in their lid-cell.—A full insight into the structure of the antheridia is only obtained when we examine them in profile. Such profile views are not seldom obtained in many accidentally-bent parts of the prothallium; we obtain them easily, also, if we fold suitably with needles prothallia which are rich in antheridia. In correct profile views (Fig. 131, *A*) we can now readily determine that the antheridium is seated

upon the middle of a slightly-arched prothallium cell (*p*), and is cut off from it by a partition membrane. The wall of the antheridium consists, almost without exception, of two tiers of superposed undivided annular cells (1 and 2) and a lid cell (3). The side-view of an emptied antheridium (Fig. 131, *B*) shows the annular cells very strongly swollen; they therefore stand out more clearly. Comparison with the surface view shows plainly their unsegmented character.

The Spermatozoids.—If we have for examination prothallia which have not been wetted for some time, we shall not have long to wait for the dehiscence of ripe antheridia. The mechanism of evacuation consists in the pressure which the annular wall cells bring to bear upon the contents, besides which a swelling substance is developed between the separated internal cells of the antheridium. The lid-cell is ultimately ruptured and the contents of the antheridium squeezed out, the annular cells then increasing in size. The contents of the antheridium come out in the form of isolated, globular cells, the spermatozoidal cells, which first lie quiescent for a short time in the surrounding water. In each cell, even with a low power, is to be recognised a coiled thread, the spermatozoid (antherozoid), and a central collection of fine granules. The walls of these cells dissolve in the surrounding water, and even in a few seconds individual spermatozoids begin to free themselves. This occurs with a jerk, whereby the coils of the body of the spermatozoid separate. One spermatozoid after another thus escapes. We follow individuals in the surrounding water, and notice that they progress comparatively rapidly, and at the same time rotate upon their axis. After about twenty to thirty minutes, the movement slackens, and finally ceases. During these last stages of the movement, the form of the spermatozoid is not difficult to recognise. This is more easily done if to the drop of water containing the spermatozoids is run in a little 10 per cent. clear filtered solution of gum-arabic, and the rapidity of their movement thus diminished. The spermatozoid (Fig. 132) is composed of a band, coiled corkscrew-wise. The coils at the anterior end are narrow, but towards the posterior become broader. The anterior

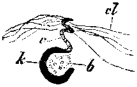


FIG. 132.—Spermatozoid of *Phegopteris* fixed with osmic acid vapour and stained with fuchsin iodine-green, and showing the position of the nucleus, *k* (× 540). Compare with Chara, Fig. 113

narrow coils bear long fine **cilia.** Between the posterior coils lies a delicate vesicle containing granules which give the starch reaction. By the addition of a little iodine the spermatozoids are very beautifully fixed. Fuller investigation shows that the posterior broader coils contain the nucleus of the spermatozoidal mother-cell.

The Archegonium.—By the anterior indentation of the prothallium, on the median cushion, we see the female sexual organs—the **archegonia.** Nearest the indentation, they are still imperfect; farther in, are ripe but unopened; still farther, perhaps dead and opened, brown inside. The female sexual organs are very easy to distinguish from the male. They project above the surface of the prothallium in the form of short, cylindrical structures, curved away from the anterior sinus. This free portion of the archegonium is only its **neck,** whilst the ventral portion is found sunk in the tissue of the prothallium. In the neck we distinguish a unilamellar **wall,** formed of four cell-rows, and a central **canal,** the contents of which, in ripe archegonia, appear granular in the central portion, and strongly refractive peripherally. This inner canal, the **neck-canal,** broadens upwards like a club. Below, it passes into the **central cell** of the archegonium, in which is found the **oosphere.** This last, it is true, is scarcely distinguishable. If the prothallia had been allowed to remain dry for several days before the commencement of the investigation, we shall probably be successful in seeing the **opening of an archegonium.** We choose for continuous observation an archegonium the contents of the canal of which appear strongly refractive. Often the opening takes place almost instantaneously; often it is necessary to wait some time. The opening of the neck is the result of the pressure which the strongly refractive swelling substance of the neck-canal exerts upon the wall of the neck. The four cells at the apex of the neck suddenly separate from one another, and the contents of the neck-canal pour out. The strongly refractive substance of this diffuses as a colourless mucilage in the surrounding water, while the granular contents are gradually disorganised. The evacuation of the contents takes place interruptedly; first come out the contents of the neck-canal, then those of the ventral canal-cell last cut off from the oosphere.

Fertilisation.—Under specially-favourable conditions we may now see the entrance of the spermatozoids into the archegonium. The chances of this are increased if we have placed with the older

prothallium, selected for the examination of the archegonia, some quite young ones, rich in antheridia. If spermatozoids are diffused in the preparation, we see them, so long as the archegonia are closed, quietly swimming by them. If, on the other hand, an archegonium has opened, the spermatozoids, from a measurable distance round, take the direction of the mouth of the canal, and are intercepted there by the mucilage. Within this mucilage their movement is slackened, but they retain their original direction; they enter into the neck-canal, and reach the oosphere, into which they are absorbed. It has been determined that here also there takes place the secretion, from the neck of the archegonium, of a substance which acts as a chemical stimulus to the spermatozoids, and determines the direction of their movement. The specific stimulant in this case is malic acid, which to the extent of about 0·3 per cent. is represented in the mass evacuated from the neck of the archegonium. Thus these spermatozoids can be successfully attracted into capillary tubes, which are fused at one end, and are injected under the air-pump with a fluid which contains 0·01 to 0·1 per cent. malic acid, combined with any base, in exactly the same way as into the neck of the archegonium. The spermatozoids of Ferns swarm into large hairs exactly as they do into capillary tubes, best of all those of the leaves of *Heracleum Sphondylium* (the Hog-weed, or Cow-parsnip), if these are laid, with their ends cut off, in water containing spermatozoids. For the spermatozoids of the mosses, cane-sugar, as we have seen, is the specific stimulant. It has been experimentally determined that a single spermatozoid suffices for fertilisation; but usually several penetrate into the archegonium, of which, however, only one finds admittance. The spermatozoids do not take their posterior vesicle with them into the archegonium; but, so far as they reach it with the vesicle still clinging to them, it is left in the mucilage in front of the opening. Now and again the number of the spermatozoids which arrive is so large that they ultimately bore in between one another, and, elongating thread-like, fill up the entire canal of the archegonium, and even still form a tuft before its opening.—The neck of the fertilised archegonium contracts rapidly at its lower end, and in from eight to ten hours commences to turn brown.

If, after completing our studies of the sexual organs, the general material is kept frequently sprayed, in from eight to ten days we can readily see the first stages of development of the

embryo in the fertilised archegonia. The venter of the archegonium has swollen, its outward-projecting wall has become multilamellar; above it stands the brown and shrivelled neck. The embryo, now composed of a larger or smaller number of cells, appears in its interior. At a later stage the wall of the venter is ruptured, and the rudiment of the first leaf makes its appearance from it.

There still remains one thing—to see the archegonia in section. *Median sections* must be taken, as the archegonia are found only on the middle line of the prothallium. In order to facilitate the cutting, we lay together several prothallia, carefully arranged one upon another, after we have previously removed all grains of sand. We now readily find the desired structures on the sections. The archegonium, as we have seen (Fig. 133, *A* and *B*), has its ventral portion sunk in the prothallium, the neck being bent. Neck canal-cell (*K'*) and ventral canal-cell (*K''*) are now distinguishable, as also the oosphere (*o*), together with its nucleus. The ventral portion of the archegonium is covered by a layer of flat cells. In a ripe, opened archegonium (*B*) a colourless spot—the **receptive spot**—can often be noticed at the apex of the oosphere, at which takes place the absorption of the spermatozoid. Less median sections may perhaps show us the antheridia, also in profile.

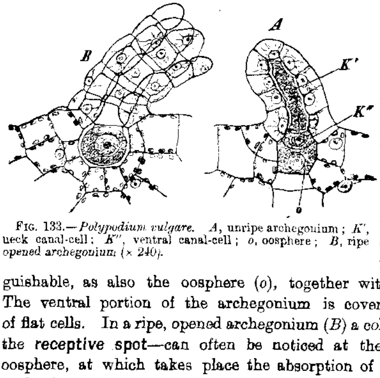


FIG. 133.—*Polypodium vulgare.* *A*, unripe archegonium; *K'*, neck canal-cell; *K''*, ventral canal-cell; *o*, oosphere; *B*, ripe opened archegonium (× 240).

Development of the Prothallium.—The raising of fern prothallia from spores has many points of interest. Amongst the quickest of all spores to germinate are those of the Royal Fern, *Osmunda regalis.* The spores of the curious annual stove aquatic fern, *Ceratopteris thalictroides*, are of special utility if obtainable, owing to the transparency of the structures developed from them. We can sow the spores on a piece of moderately soft tile, laid

in water in a saucer, or upon a flower-pot or flower-pot saucer similarly kept constantly moist. In a room it may be covered over with a bell-globe. In this way all the early stages of development can be well obtained, and it needs only to scrape off some of the germinating spores day by day with the blade of a pocket-knife, and lay them in water on an object-slide, to be able to follow the development.—For full-grown prothallia for section-cutting, the spores can be well sown on a bed of cocoa-nut fibre refuse, flattened down in a large flower-pot saucer, with a hole in the bottom, or a seed-pan, and well drained, kept moist until towards the time they are needed for examination. Over-head watering, if needed, can be given with a spray such as is used for diffusing scent, and by gardeners for the purpose of spraying foliage with insecticides.—Another highly-recommended method is to sow the spores upon a slab of peat which has been first boiled in water in order to kill any seeds or spores it may contain, and then soaked with the culture fluid recommended for the cultivation of *Spirogyra.* The spores are then sown, and the turf covered with a bell-glass and placed in a north window. With a favourable temperature germination begins in from three to five days.—With care to keep them moist, the spores can likewise be germinated upon a glass-slide, and, with the very careful addition of minute quantities of culture fluid, can be grown to some size, though their growth thus is usually much more slow. Owing to their large size, the spores of *Ceratopteris* lend themselves pretty readily to this mode of culture, which enables easy observation under the microscope, and facilitates also the fixing and permanent preservation of specimens showing the early stages of germination and prothallial development. The spores of *Ceratopteris* can likewise be germinated by sowing upon a nutrient fluid, and preserving a moist atmosphere. Floating prothallia are then developed, with rhizoids and sexual structures in their normal position, but submerged.—The spores of various kinds of *Gymnogramme* (widely grown as "silver" and "gold" ferns) are also exceedingly good for growing prothallia. With young prothallia spermatozoids can be very well studied in suspended drops in moist chambers, and the nature and duration of their movement, and the changes accompanying it, readily followed.

Reproduction of Equisetum.—The sporangiferous axis of *Equisetum* arises in the form of a cone, at the tip either of the ordinary

green, or of special colourless shoots. The sporangiophores are metamorphosed leaves, arranged in whorls, and, by mutual lateral pressure, have assumed a polygonal, usually hexagonal, form. In order to study them more closely we first remove a number of them with a scalpel from the axis of a ripe sporangiferous shoot, and examine them dry, with direct illumination, under the simple microscope. All species are practically alike; we will, however, select the common *Equisetum limosum* of ditches, fructifying in June and July.

In each **sporangiophore** it is easy to distinguish the polygonal shield and the stalk which bears it. On the inner surface of the shield, surrounding the stalk, arise about eight sac-like **sporangia**, which, in order to evacuate their spores, split along the entire length of the side directed towards the stalk.—The internal structure of the sporangiophore and the sporangia can be seen upon *cross-sections*, taken through an axis which is not quite ripe. Decidedly more favourable results will be attained by the use of alcohol material, softened and examined in glycerine. The stalk of the sporangiophore is traversed by a central vascular bundle. At its apex it broadens out into the shield, and its vascular bundle divides, umbrella-wise, into just so many strands as there are sporangia present. The bundle-branchlets end with spirally-thickened tracheïdes under the place of insertion of the sporangia. The epidermis of the sporangia is distinguished by the beautiful spiral, or in part annulär, thickening of its cells. The sporangial wall appears reduced to this epidermis, and to some ultimately-collapsed layers of cells. During their formation the spores lie embedded in an epiplasm, very rich in starch, and fixed by alcohol, which is consumed during the later development of the spores.—We will study the **spores** of *E. limosum* in fresh material. These are distinguished at once by their striking **elaters**. These elaters are two bands, derived from the cleft outer membrane of the spore. They hold together only at one spot, in rectangular fashion, and form therefore a four-armed cross, whose arms are rolled around the spore. At their end, the bands are expanded spathulately. These bands are very hygroscopic; upon drying they unroll, in moist air they roll up again. If we breathe upon dry spores lying (uncovered) upon an object-slide, and while observing them under the microscope, the bands will be seen to begin to roll up, whereby the whole mass of spores is set in movement. The use of this mechanism lies in the hook-

ing together of the spores, which brings about a gregarious development of the unisexual prothallia, and hence enhances the probability of fertilisation. Besides the membrane which gave rise to the elaters the spores possess two other smooth membranes, in close contact, and forming together, apparently, only a single wall. In alcohol material the two membranes separate, and can be readily seen. With a correctly-disposed spore it can be seen that they are joined at one spot. At the same time the median nucleus of the spore is clearly distinguishable. If we run chlor-zinc iodine into such a preparation, the elaters take a dirty violet colour—only, however, in their inner parts, while the outer become brownish; the median membrane stains yellow-brown; by it this membrane becomes wrinkled. The contents of the fresh spore appear green from the presence of numerous small chlorophyll bodies.

Reproduction of Selaginella.—The **Selaginelleæ** are **heterosporous** Lycopodineæ; they possess two kinds of sporangia and spores, and we will therefore turn our attention to them in order to broaden our view of the Vascular Cryptogams. The Selaginelleæ are also known as the **Ligulatæ**, because their leaves are provided with a small ligule at the base. We will examine more carefully *Selaginella Martensii* (Sprg.), universally distributed in plant-houses. Fertile specimens are easy to recognise by the short spikes which they usually develop on the ultimate branches of numerous shoots. The vegetative body of the plant is spread in one plane; it bears four rows of leaves in pairs, which cross one another obliquely. In each pair the upper leaf remains small, the under is considerably larger. The two rows of upper leaves on the dorsal surface press against the stem with their upper side. The two rows of under leaves on the ventral surface are placed laterally, flatly spread out, with their upper surface upwards. The vegetative body of the plant is therefore **bilateral** and **dorsi-ventral**; that is, it admits only one plane of symmetry, which divides the body into a right and left half, and exhibits a ventral and dorsal surface. The terminal **fertile spikes**, on the other hand, are quadrangular, provided with four rows of symmetrically arranged leaves, directed outwards.—We next inform ourselves as to the structure of the spike, by pulling off one leaf after the other with needles under the simple microscope, beginning at the base. We see an ovate, somewhat-flattened **sporangium** stand in the axil of each leaf. Even in this operation we shall have noticed that many sporangia are larger, and show projecting bosses. If

we open these larger sporangia with the needles, four large spores will come into view, which completely filled the sporangium, and made its wall bulge locally; if we open one of the small sporangia, it proves to be filled with numerous small spores. The large sporangia are female sporangia (**macrosporangia**), the large spores female spores (**macrospores**); the small sporangia and spores are male, and are distinguished respectively as **micro-sporangia** and **microspores.** The small spores are triangularly pointed on one side, with reticulate markings, and usually hold together in tetrads. The same relations, more evident in accordance with size, are met with on the four macrospores. We see clearly upon them the triangular tapering of one side; in order, on the other hand, to be able to distinguish well the reticulately-connected ridges on the cell-wall, it is desirable to crush the spores. The walls of the microspores soon become dark brown, while the macrospores remain far paler. If we examine the leaves, from which we have removed the sporangia, we see the **ligule** arise close under the place of insertion of the removed sporangium, as a tongue-shaped membrane. A further removal of leaves from the spike shows us that the macrosporangia are far scarcer upon it than the microsporangia, but preponderate on the lower parts of the spike. The ripe sporangia dehisce transversely into two valves.

Between thumb and forefinger we will now prepare *median longitudinal sections* both of the apex and of the lower part of the spike, holding the material in such way that the section passes through the middle line of a pair of leaves. In favourable sections, taken from spikes still growing, we have now before us the entire developmental history of the sporangia, from their rudiments to the ripe condition. The apex includes the growing point with the youngest leaf and sporangial rudiments. At the growing point we may indeed be able to see the two- or three-sided tapering apical cell. We see the sporangial protuberances originate over the youngest leaf rudiments. We note the early origin of the ligule close below the sporangial protuberance at the base of the leaf. Also we may see various stages in the development of the macro- or microspores from a central cell in the sporangium, and of the sporangial wall.

In conclusion, it may be mentioned that the Selaginelleæ, in drying, preserve so excellently, that we can use softened herbarium specimens in order to study the growing point and the origin of the sporangia. Sections through fresh material, as well as material thus softened, can be made very transparent with potash solution.

CHAPTER XXVII.

THE REPRODUCTION OF GYMNOSPERMS.

MATERIALS USED.

Male flowers of *Pinus* (*e.g.*, the Scotch Fir, *P. sylvestris*). Best in alcohol. May or June. Also fresh.

Young female cones of the Scotch Fir (or other *Pinus*). Fresh, or in alcohol. End of May.

First year cones of the Red Fir (*Picea vulgaris*, Lk.). Weekly from end of May to end of June. Fresh, or best in alcohol.

Seeds of the same. September or October.

Alcohol material, whenever possible, is preferable to fresh, as the resin will be dissolved out. It should lie for at least twenty-four hours before use in alcohol-glycerine.

REAGENTS USED.

Potash—Iodine—Carbolic acid.

PHANEROGAMIC plants fall into the two great divisions of **naked seeded**, or **Gymnosperms**, and **enclosed seeded**, or **Angiosperms**. These divisions differ greatly in the structure of the flower and the processes of fertilisation and embryology, and we will first study them in the Gymnosperms.

Male Flower of Pinus sylvestris.—We will make ourselves acquainted in the first instance with the structure of the **male flower** of the Scotch Fir (*Pinus sylvestris*). This plant flowers in May or June, according to the district; but it can be studied very well in alcohol material, which, as it is too brittle, should be laid for at least one day prior to examination in a mixture of equal parts of alcohol and glycerine. Material thus prepared can be cut much better than if fresh. We first determine that the male flowers (in the form of tiny cones) stand in large numbers on the lower parts of a shoot of the same year. They are arranged according to a $\frac{5}{13}$ phyllotaxy, and correspond in their arrangement exactly to the "**short shoots**," each bearing two needle

leaves, which succeed the flowers in uninterrupted series. The flowers also, like the short shoots, stand in the axils of **scale-leaves** or **bracts.** Upon the stalk of the male flowers, we find first three decussating pairs of bracts. The lowermost pair is placed laterally with regard to the primary bract and the parent axis, an arrangement which is due to the necessities of space, and which is almost always found in the first pair of leaves of the vegetative buds of Gymnosperms. To the bracts of the short flower-stalk succeed the **stamens,** closely crowded, usually ar-

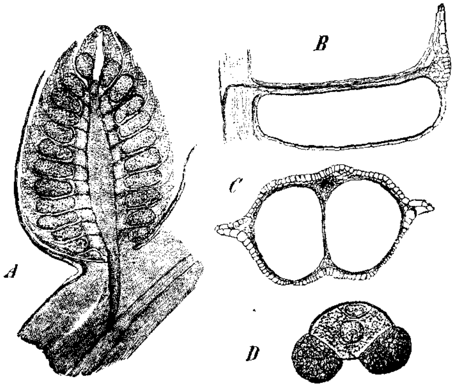


FIG. 134.—*Pinus Pumilio*, resembling *P. sylvestris*. *D*, from *P. sylvestris*. *A*, longitudinal section through a nearly ripe male flower (× 10). *B*, longitudinal section through a single staminal leaf (× 20). *C*, cross-section through a staminal leaf (× 27). *D*, a ripe pollen-grain (× 400).

ranged in ten vertical rows. The floral axis is elongated, fusiform. A single stamen separated and examined under the simple microscope shows its under side occupied by two longitudinally-inserted **pollen-sacs,** meeting one another in the middle line; its tip is extended into a short, upturned edge.

Median longitudinal sections through the flower, shortly before the dehiscence of the anthers (Fig. 134, *A*), show, especially after treatment with potash, the course of the vascular bundles in the floral axis, the series of staminal leaves, each with a single

vascular bundle, the insertion of the pollen-sacs on the staminal leaves. Upon fragmentary longitudinal sections, thinner parts can be readily found, in which the structure of the individual staminal leaf (*B*) may be still better followed.

We next prepare *tangential longitudinal sections* through the flower, in order to obtain cross-sections of single staminal leaves, and pick out such an one for closer study (*C*). We see that the two pollen-sacs adjoin in the middle line, and, when ripe, are usually separated only by a flat wall of collapsed cells, in which one or more layers of flat starch-containing cells may be medianly interposed. Upon their free outer surface the pollen-sacs are covered by the epidermis, to which, towards the interior, usually only collapsed cells adjoin; towards the dorsal surface of the leaf, likewise, the pollen cavities are enclosed in the same way. In the median line of the anther, above and below the partition wall separating the two pollen-sacs, runs a strip of mesophyll. The upper of these is thicker, and is traversed by the very delicate vascular bundle. At the two side edges of the anther, the epidermis projects into a more or less feebly-developed wing; where this is thickest, a little mesophyll can be found between the two layers of epidermis (*C*). On the under side of the pollen-sacs the epidermal cells diminish in size from both sides; at the places of weakest development the pollen-sacs open. These pollen-sacs closely resemble the sporangia of Lycopodiaceæ; researches in comparative development have, in fact, led to the conception that the **pollen-sacs** of Phanerogams, and the **microsporangia** of Cryptogams are homologous structures, and that the pollen-grains, therefore, are microspores.

The Pollen-Grain.—If we look now to the **pollen-grains** developed in the pollen-sacs, where possible in the fresh state, we shall note that each of these consists of a central body, upon which are placed laterally two bladdery vesicles (*D*). If the flower is ripe, the two vesicles appear dark, because filled with air. They show a delicate tracery upon their surface. The middle, true pollen-cell, contains finely granular protoplasm, and a large nucleus. Shortly before dehiscence, *i.e.*, before the opening of the pollen-sacs, a division takes place in the pollen-grain, and in the ripe pollen-grain we see, in the middle of the convex side, turned away from the two vesicles, a lenticular cell adpressed to its wall. This cell is best seen when the pollen-grain, as in our figure, lies on its side. In the pollen-grains of

most Coniferæ several such cells adjoin one another, and form a characteristic cell-body projecting more or less deeply into the pollen-grain. These internal structures in the pollen-grain of Gymnosperms are to be looked upon as a **reduced prothallium**, which in *Pinus* is reduced so far that only a single cell, besides the generative cell, remains in the ripe pollen-grain. The pollen-grain of *Pinus* has a double membrane, the outer, the **exine** (or **exospore**), the inner the **intine** or (**endospore**). The exine is cuticularised, and at two laterally-situated places has undergone cleavage in order to form vesicles, which first contain fluid, but when ripe are filled with air, and thus form these bladdery wing-like appendages.

Female Cone of Pinus sylvestris.—The Scotch Fir is monœcious, so that we find male and female flowers upon the same plant. The **female flowers** form a **cone**, in which numerous ovules, inserted upon scale-like structures, are found combined. The small cones, either singly or several together, occupy the apex of twigs of the same age as themselves. They stand in the axils of bracts just like those of the short shoots, each with two needle leaves, inserted lower down on the axis; their position at the end of the shoot corresponds, however, with that of the normal twig-forming prolongation. The small cones are usually in the receptive state at the end of May, and, though small, are recognisable by their brown-red colour. They are stalked, and stand erect; the stalk is covered with brown scales. Here also alcohol material treated with glycerine can serve for study. If we bring pieces removed from the axis of the cone with the scalpel under the simple microscope, and isolate them with needles, we can see (Fig. 135), that in the axils of delicate obovate **bracts** (*b*), somewhat fringed at their margin, arise scales (*fr*) of similar form, but fleshy, smooth edged, provided on the inner side with a central projecting rib (*c*). These are distin-

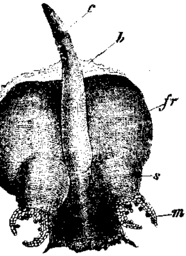


FIG. 135.—*Pinus sylvestris*. Fruit-scale *f* with its two ovules, *s*, and the central rib, *c*. Behind is the bract, *b*. Upon the ovule the integument has grown out into two prolongations, *m* (× 7).

guished as **fruit-scales.** At the base of the fruit-scale, right and left, is found on each side of the rib an **ovule** (*s*), with its **micropyle** turned below and towards the outer side. The edge of the simple **ovular integument** is prolonged at the micropyle into two lobes (*m*), placed right and left. Bract and fruit-scale have grown together at the base, and are therefore removed together from the axis of the cone.—The cones of the Abietineæ, and other true cone-bearing Coniferæ, are conceived to be either single flowers or inflorescences, according to the significance which is attached to the fruit-scale. This may either be considered as a flattened metamorphosed axial shoot, growing in the axis of a modified leaf, which we have here called the bract, and partially adnate to the bract; or, as a development of the placenta of a carpellary leaf, which we have hitherto called the bract. In the former case, therefore, we should have a bi-ovular branch in the axil of each bract; in the second a bi-ovular placenta on the upper side of a carpellary leaf. In the former case, the cone would therefore be an **inflorescence** composed of many fertile axillary branches; in the second, the cone would be a **single flower** composed of numerous open carpellary leaves.

Pollination.—The remarkable structure of the fruit-scale is explained by the machinery for **pollination**, which can be followed upon fresh material only, and at the time of pollination. As soon as the male flowers begin to free their pollen (which is carried off by the wind), we can demonstrate an elongation of the axis of the cone, whereby the fruit-scales, together with the bracts appertaining to them, are separated from one another. The pollen can now fall upon the uplifted fruit-scales, slip down them, and guided by the projecting rib, come between the two prolongations of the ovular integument. Later on, these prolongations curve inwards, and in this way carry the pollen into the micropyle, and to the apex of the **nucellus.** After full pollination the fruit-scales soon close their edges together, and these are consolidated by the intergrowth of papillæ. The bracts do not further develop, nor does the central rib of the fruit-scale, which is of no further use. The red colour of the cone changes into brown, and then into green (when ripe, again becoming brown); and the cone slowly bends over, and finally takes a pendant position.

The Ovule and Embryo-sac.—We will now turn our attention to the further changes which take place in the pollinised ovules of the Coniferæ. At the time of pollination the **embryo-sac** of the

ovule is in a most rudimentary condition, and is not easy to find; longitudinal sections through the ovule shows the **nucellus** as a small protuberance surrounded by the simple integument. After this a further development of the ovule takes place, and at a rate which depends upon the greater or less time which has to separate the periods of **pollination** and of **fertilisation.** In *Taxus* (the Yew) fertilisation takes place about the middle of June in the same year; in the Scotch Fir not until the next year, more than thirteen months after pollination, and in this case the further development of the ovular rudiment begins in the spring following pollination, and must therefore be studied in one year old cones. In the Spruce Fir (*Abies*, *Picea*), pollination and fertilisation are separated by about six weeks only, and the further development of the ovular rudiment follows close upon pollination. We will consequently, in what follows, keep to the Spruce Fir, because this offers many advantages for study. It would lead us too far to follow step by step in our present studies the enlargement of the **embryo-sac**, the origin of the tissue of the **prothallus** (**endosperm**) and of the sexual organs in its interior, the increase in size and corresponding differentiation of the entire rudimentary seed. We will therefore turn at once to the stage in which the **oospheres** are fully formed, and in a receptive condition. This condition, in the common Red Fir (*Picea vulgaris*, Lk.),[1] is reached about the middle of June, and fertilisation is then completed in the course of a few days. Alcohol material in suitable stages of development must be at command. It is recommended not to lay entire cones, but separate fruit-scales, in the alcohol. Before cutting the alcohol material, it should be transferred, as we have already repeatedly done, to a mixture of equal parts alcohol and glycerine for at least twenty-four hours.

In beginning the investigation, we first study the appearance of the entire scale. This is obovate, shows below, on its inner (upper) surface, the two ovules, and also the outlines of the membranous wings, which later on will be separated, with the ripe seed, from the inner surface of the fruit-scale. On the outer surface of the fruit-scale, and below, can still be found the bract, now appearing comparatively minute.

The ovule to be out can be easily separated uninjured from the

[1] *Picea vulgaris*, Link (1827), of the text, was renamed by the same author 14(8)1 *Picea excelsa*. It is *Pinus Picea* and *Abies Picea* of various authors.

fruit-scale with the points of the needles. We prepare *longitudinal sections* of it between thumb and forefinger, but as the integument has become comparatively hard we must somewhat modify our method of preparation. We cut the ovule in two with the scissors at about its mid height; we then take the upper half of the ovule, *i.e.*, that which contains its apex, betweeu the fingers, and with the forceps withdraw the nucellus out of the cut surface. Through this longitudinal sections can now be readily made. We examine in glycerine. — We first, however, examine a longitudinal section of an entire receptive ovule with a low power. The entire ovule, with integument, is cut perpendicularly to its surface of insertion; it is displayed, therefore, in median longitudinal view (Fig. 136). We see in it the **integument** (*i*), which develops into the skin of the seed, and from mid-height is separated from the nucellus; the **nucellus,** bearing upon its apex **pollen-grains,** which partly are external, and partly lie sunk in its tissue; or may even show **pollen - tubes** (*t*), developed from these pollen-grains, which penetrate the upper part of the nucellus, in order to reach the external layer of the embryo-sac, the **embryo-sac** (*e*), of elliptic outline, filled with **endosperm** (or more correctly, prothallium tissue); the **archegonia,** of which the ventral portion (*a*) is very large and proportionately easy, but neck more difficult, to recognise; in the interior of each arche-

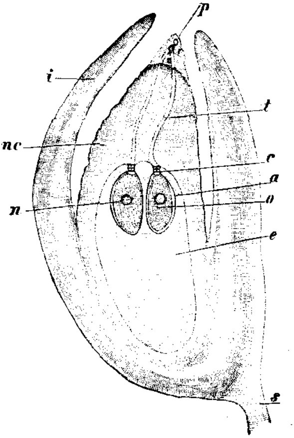


FIG. 136.—Median longitudinal section through a receptive ovule of *Picea vulgaris*, Lk. *e*, embryo-sac filled with endosperm; *a*, ventral portion, and *c*, neck of an archegonium; *n*, the nucleus of the oosphere; *nc*, the nucellus of the ovule; *p*, pollen-grains upon and in the nucellar apex; *t*, pollen-tubes, traversing the nucellus; *i*, integument; *s*, the wing of the seed (× 9).

gonium is an oosphere (*o*), which in alcohol material is noticeable from its yellow-brown colour, and shows a central large nucleus (*n*); and lastly, under the ovule, the commencement of the wing (*s*).—If we prepare a similarly-directed section through a *fresh* ovule of the same age, we shall again find the same relations; but very commonly the contents of the archegonium will have run out. If the section has laid bare individual archegonia, without opening them, the oospheres will appear as yellowish frothy masses of protoplasm, in which the central nucleus is scarcely distinguishable, or else, in the best cases, has only the appearance of a large central vacuole. The oospheres quickly suffer under the influence of the water taken from their environment; if the section is to be kept for a longer time, it is recommended to use as fluid for observation white of egg diluted with water, to which, for greater stability, a little camphor has been added. In such preparations the neck of the archegonium is not difficult to see. It consists of from two to four stages of cells. Under the neck is to be found a small cell (Fig. 137, *A*, *cl*), which corresponds with the ventral canal-cell of the Vascular Cryptogams; the oosphere divides, in order to form it, shortly before it is receptive. The venter, or body, of the archegonium is surrounded by a layer of flattened cells, rich in cell-contents.—In order to inform ourselves as to the number and position of the archegonia, we prepare a number of successive cross-sections through the upper part of the ovule. In this way we show that from three to five archegonia, arranged in a circle, stand at the apex of the embryo-sac. Sections which have laid bare this apex show us the neck of the archegonia, in apical view, as rosettes of six or eight cells.

Fertilisation.—If our material has been gathered at the time of fertilisation, we may be able to follow individual pollen-tubes to an oosphere. As longitudinal sections show, the pollen-tubes penetrate to the embryo-sac through the conducting tissue of the nucellus, growing in that direction in which they are best nourished. The pollen-tube is thickly filled with small grains, which iodine shows to be starch. In suitably fixed material one can without difficulty see the reproductive cells of the pollen-grain, and the vegetative nucleus, in the swollen end of the pollen tube. The reproductive cells are present to the number of two, which have resulted from the division of their mother-cell. The pollen-tubes attain to the embryo-sac, penetrate between the cells of the neck, and thus to an oosphere, into which one of the two

reproductive cells passes (Fig. 137, *B*). In specially fortunate sections one may see the smaller spermo-nucleus pressed against the larger oonucleus (Fig. 137, *C*), and then the two unite into the embryo-nucleus. The apex of the pollen-tube in *Picea* is finely

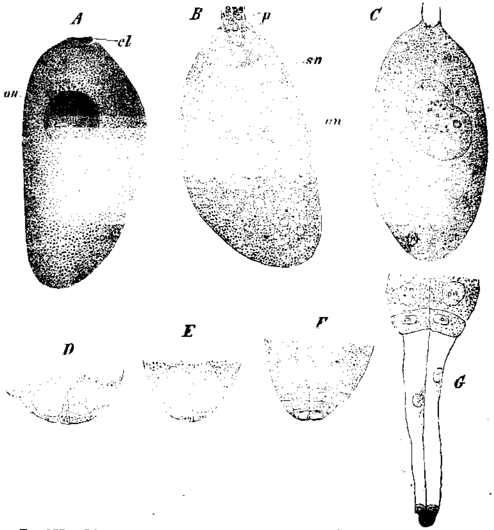


FIG. 137.—*Picea vulgaris*, Lk. *A*, a ripe oosphere with nucleus, *on*, and ventral canal-cell, *cl*. *B*, an oosphere during fertilisation; *sn*, the intruded spermo-nucleus; *on*, the oonucleus; *p*, the tip of the pollen-tube. *C*, an oosphere during fertilisation, showing the conjugation of spermo- and oo-nucleus. *D*, the four nuclei in the end of the oospore remote from the neck, only two of which can be seen, since they all lie in the same plane. *E*, the nuclei have divided; four nuclei lie now at the end, and four others more in the body of the oospore. *F*, three stages of cells are formed in the end of the oospore. *G*, the middle stage has elongated, and carried the lowest stage into the endosperm. The cells of the lowest stage are specially rich in contents (× 90).

porous, and in species of *Pinus* shows a clear pit, which, however, does not suffice for the passage of the nucleus; but still the membrane at the tip of the pollen-tube is soft and swollen, so that it would offer no great resistance to the passage of the nucleus.

The reproductive nucleus which passes into the oosphere has therefore the value of a spermatozoid, and indeed in *Zamia*, *Ginkgo biloba* (*Salisburya adiantifolia*), and *Cycas revoluta*, all Gymnospermous Phanerogams, large multiciliate spermatozoids have actually been discovered. In general we may say that the reproductive nucleus of the pollen-tube is distinguished only in the details of form from the spermatozoid of a Vascular Cryptogam, while the want of motility is compensated for by the growth of the pollen-tube right up to the embryo-sac. In both cases the nucleus forms the main body of the fertilising mass.

Development of the Embryo.—The next stages take the embryo nucleus into the end of the oospore remote from the neck, where, by repeated bipartition, it forms four nuclei lying in the same plane (Fig. 137, *D*). These are separated from one another laterally by partition walls. They repeat their bipartition transversely to the long axis of the oospore, and become separated off from one another in this direction also (*E*). The four nuclei lying at the end of the oospore again divide in the same direction, and the resulting nuclei which now lie nearest to the end of the oospore once more divide. Ultimately therefore, at that end of the oospore which is farthest from the neck, we find three tiers, each of four cells, and above these, in the general body of the oospore, four free nuclei (*F*). These free nuclei enlarge very considerably, and later on sink. Of the three tiers of cells, that which is nearest to the neck remains as a four-celled rosette at the base of the archegonium, the median ones elongate, forming the "embryonal tubes," from which the **suspensor** is formed, and carry the cells which were most remote from the neck of the archegonium down into the tissue of the prothallium (*G*). These last cells constitute the rudimentary **embryo.** They are distinguished from the beginning by their richer contents, and soon divide into two (so early as *G*), and then into three stages.

We can use the same fir, *Picea vulgaris*, Lk., in order to study older ovules with rudimentary embryos. We can either use the material fresh, and at intervals of about eight days, or the material can at similar intervals be laid in alcohol. The rudimentary embryo rapidly increases in size and in number of cells by the formation of periclinal, anticlinal, and radial walls, and takes the form of the adjoining Fig. 138, *A*. These divisions preclude from the very first the existence of an apical cell. After the embryo has further enlarged, its hinder end begins to develop

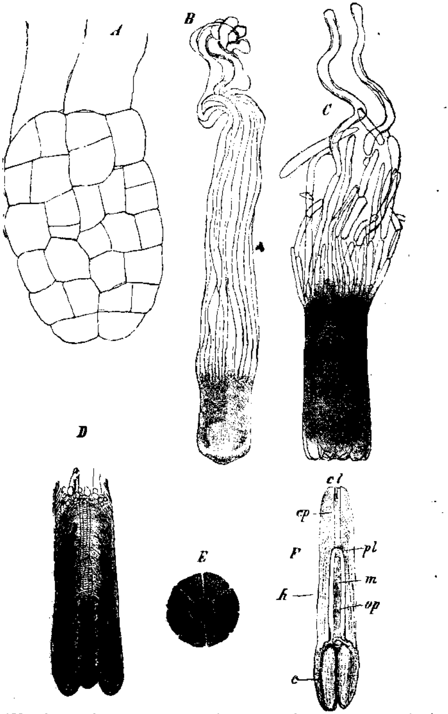


FIG. 138.—*Picea vulgaris*. *A*, young rudimentary embryo in optical section (× 240). *B*, older rudimentary embryo in optical section. The rudimentary root and growing point of the stem are already formed (× 27). *C*, half-ripe embryo seen from the outside. *D*, the same in longitudinal section. *E*, in apical view (× 27). *F*, longitudinal section through a ripe seed, showing: *c*, cotyledons; *h*, hypocotyl; *pl*, apex of the plerome of the root; *cp*, root-cap; *cl*, its central column; *m*, pith; *op*, procambial ring in the hypocotyl (× 10).

sac-wise, and adds to the embryonal tubes, so that the "suspensor" formed from these becomes more and more massive. The rudimentary embryo itself assumes a cylindrical form, becomes opaque, and is then very sharply contrasted with the transparent suspensor. When the opaque portion has attained a length of about 0·5 mm., the rudiment of a root can (after the use of potash, creosote, or chloral hydrate has made it transparent) be made out in its interior. This is differentiated at about 0·15 mm. distance from the apex and always by periclinal divisions within a layer of hemispherically-arranged cells (Fig. 138, *B*). Henceforth this root apex adds to the length of the embryonic axis posteriorly by its growth. Soon the other end of the embryonic axis bulges in its middle portion (*B*), in order to form the growing apex of the stem. Around this then arise in considerable number the rudiments of the seed-leaves or **cotyledons** (*C*, *D* and *E*). All the parts of the embryo are now present, and need only to grow in order to attain the structure visible in the ripe seed.

Thus far we have concerned ourselves solely with the most strongly-developing embryo, which ultimately is alone present; but in reality several, if not all, of the archegonia give rise to rudimentary embryos. All these rudiments grow back into the long axis of the body of the prothallium; that one, however, which originates before the others, and first, therefore, makes use of the food-materials stored in the tissue of the prothallium, develops more strongly, and ultimately supplants all the others. At the time when the cotyledons show themselves, the rudimentary embryo already lies with its apex at the base of the embryo-sac. With further growth the root end must now be again extended backward, and ultimately attains the position whence the whole development started. The suspensor is pressed upwards, and ultimately reduced to a knot of disordered cells. The individual cell-rows constituting it separate easily from one another (*C*).

The Seed.—The **seed** ripens in October. It then easily separates from the fruit-scale, together with the wing which is developed on the inner side of the seed, leaving behind upon this fruit-scale, after separation, a corresponding hollow. The cells of the **spermoderm** are, as cross and longitudinal sections readily show, thickened almost to the obliteration of their cavity. A portion of the tissue of the prothallium remains in the seed.

as albumen or endosperm, densely filled with reserve food-materials. It forms a sac, enclosing the embryo. This sac is open at its micropylar end, and here the radicle of the embryo is situated against the displaced remnant of the nucellus. The embryo can be easily made out in seeds cut in the direction of their length. It looks somewhat cylindrical, gradually getting thicker towards the cotyledonary end. In consequence of being filled with reserve food-materials it is white, and as opaque as the albumen or endosperm of the seed.

We prepare a *median longitudinal section* through the seed between the fingers, and lay it in carbolic acid diluted with alcohol. The figure becomes very beautifully clear, far better than in potash, and even better than in chloral hydrate, so that we can follow every row of cells. We see (Fig. 139) that the cotyledons (*c*) do not reach quite a third of the whole length of the embryo. At the base between them is to be seen the growing apex (*punctum vegetationis*) of the embryonic stem. The stem (caulicle) itself, which is distinguished as the hypocotyl (*h*) passes, without clear limitation, into the root (the radicle). This is for the most part represented only by a growing apex, which shows clearly in the interior of the body of the embryo as the apex of the plerome (*pl*) of the root, while the cell-rows of the cortex (periblem) of the hypocotyl pass directly into the parabolic layers of the rootcap (*cp*), a condition which recurs in all roots of the Gymnosperms, since we can see the cell-rows of the cortex of the body of the root pass over direct into the cell-layers of the root-cap (*cf. Thuja*, p. 223). The root-cap is traversed in the direction of its long axis by a distinctly-marked column of tabular cells, arranged in straight rows. In the hypocotyl the tissue of the pith (*m*) already begins to show, and around this the elongated cells of the procambium ring (*op*), in which the vascular bundles will make their appearance. These cells can be traced, moreover, for a short distance along the median section of the cotyledons (compare the Fig.). Thus in the embryo the essential parts of the future plant are already present.

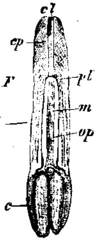


FIG. 139.—Longitudinal section through the ripe embryo. *c*, cotyledons; *h*, hypocotyl; *pl*, growing apex of the plerome; *cp*, root-cap; *cl*, its central column; *m*, pith; *op*, procambium ring in the hypocotyl (× 10).

CHAPTER XXVIII.

THE ANDRŒCIUM OF ANGIOSPERMS.

MATERIALS USED.

Flower-buds of various ages, and flowers, of the Day Lily (*Hemerocallis fulva*); fresh, or in alcohol. Summer. In its stead, species of *Lilium* Tulip, Hyacinth, *Funkia ovata*, *Agapanthus umbellatus*. *Iris*, etc., thus providing material available during a great part of the year.

The same of *Tradescantia virginica;* fresh. Or the Snowflake (*Leucojum*), or *Fritillaria imperialis* (the Crown Imperial).

Flower-buds, about to open, and older flowers of *Œnothera*, or *Fuchsia*, or *Epilobium ;* fresh.

Flowers of *Althæa rosea*, or other Malvaceæ; fresh, also in alcohol.

Flowers of *Calluna*, *Erica*, *Azalea*, *Rhododendron* or *Acacia* species; fresh.

Flowers for pollen-cultivation; fresh.

REAGENTS USED.

Concentrated sulphuric acid—Chlorzinc iodine—25 per cent. chromic acid —Acetic iodine green—Iodine—Logwood—Carbolic acid—Chloral hydrate—Oil of cloves—Oil of lemon—Iodine green—Acetic acid—Sugar solution with gelatine.

THE male sexual organs of an **Angiospermous flower** form collectively the **andrœcium.** The individual **stamen** consists of a (usually) thread-like stalk, the **filament,** and the **anther.** This last is formed of two longitudinal halves or **anther-lobes,** which are separated by the upper part of the filament, the so-called **connective,** but the connective is reckoned as a part of the anther. In the tissue of each anther-lobe are contained usually two compartments, or **pollen-sacs.** Each pollen-sac corresponds with a **microsporangium.**

Stamen of Hemerocallis.—We first inform ourselves about the stamen of some one of the large-flowered Liliaceæ; for example, *Hemerocallis fulva*, a hardy herbaceous perennial, very widely cultivated in gardens, or any of the still more universally

cultivated white or tiger Lilies, Tulip, Crown Imperial, etc., will do equally well. The yellow filament is here very long, becomes thinner towards its upper end, and tapers very sharply at the place of insertion of the anther. This latter is brown in *Hemerocallis*, and movable (versatile or centrifixed) upon the filament. The connective can be traced along the outer side of the anther as a thin stripe between the two anther-lobes.

The Pollen-Grain.—The ripe **pollen**, observed dry upon the object-slide, shows the form of coffee berries, the general form in Liliaceæ. It appears yellow, ornamented with a network of ridges on its surface. If, while examining, we allow water to enter from the edge of the cover-glass, we see that each pollen-grain, as soon as wetted, levels up its furrow, strongly bulges out on this particular side, and takes the form of a unilaterally flattened ellipsoid. The membrane of the previously-furrowed part is without ridges, is thick and colourless, and is delimited sharply against the sculptured, brownish membrane. This brown membrane is the outer wall of the pollen-grain, the **exine** (or **exospore**); the swollen colourless membrane is the inner, the **intine** (or **endospore**). In the swelling of the pollen-grain the exine has been ruptured in the grooved side, and the strongly-thickened intine protrudes. On the opposite side the intine is present only in the form of a delicate skin. The exine subserves the protection of the pollen-grain, and completely covers it in its normal furrowed condition. Between the pollen-grains in the preparation orange-red oil is distributed, and it clings also to the surface of the grains, giving to them in the dry state their yellow colour. The contents of the pollen-grain appear grey and finely granular. After a short time, during which the pollen-grain slowly and progressively enlarges, it bursts and empties its contents, in worm-like form, into the surrounding water. In sugar solution of suitable concentration the grains round off without bursting, and can be examined uninjured.

If we allow concentrated sulphuric acid to act upon the pollen-grains, the colourless smooth part of their wall is at once dissolved, while the sculptured, brownish part, on the other hand, resists: it is cuticularised. If into a preparation of pollen-grains in water we run watery solution of methylene blue, the cuticularised exine assumes a faint greenish-blne colour, the swollen intine bright blue, somewhat shading into violet. If instead of methylene blue we use watery safranin solution, the exine is

stained cherry-red, the intine orange-red. The bright blue of the former and orange-red of the latter stain are characteristic of pectic substances, which latter constitute the greater proportion of the intine. The intine in this case is so rich in pectic materials that with chlorzinc iodine it gives well only the bright yellow pectic reaction, and at most its innermost homogeneous layer alone shows an indistinct cellulose reaction. The intine of pollen-grains always contains pectin, apart from which the cellulose reaction is easy to obtain in it. The exine, as always with cutinised membranes, stains yellow-brown with chlorzinc iodine.—Alcohol material of the pollen of *Hemerocallis fulva* reacts even better than when fresh. The oil is wanting in such preparations, since it is removed by alcohol. In the outer portions of the intine addition of water brings out striæ perpendicular to the surface. Sulphuric acid makes the structure of the cutinised exine still clearer, and high magnification shows a delicate sinuous network. The acid has turned the yellow oil masses blue, and they lie as irregular bodies in the meshes of the exine. The cutinised membrane itself has become yellow; after some hours it becomes reddish-brown, while the extruded contents of the pollen-grain are stained rose-red, a reaction which protoplasmic substances show under the influence of sulphuric acid and in the presence of sugar.—In 25 per cent. chromic acid the non-cutinised parts of the exine, and the contents of the pollen-grain, are quickly dissolved; the cutinised parts resist its action longer. In its comparatively ready solubility in chromic acid the substance of the exine differs from corky and cutinised cell-walls, but it is nevertheless closely related to suberin and cutin.

Structure of the Anther Wall.—We will now prepare cross-sections through the anthers; and first it would be well to turn to a flower-bud only about two-thirds grown, and cut *cross-sections* through this. The sections of the perianth are then removed from the preparation with the needles. Although we have chosen so young a flower for investigation, we nevertheless find all the pollen-sacs open. Their opening is effected very easily, and in this case by the pressure of the razor in cutting. The adjoining figure (Fig. 140, *A*) will help us to understand the structure. The walls of the pollen-sacs break away (at *p*) from the partitions separating the two sacs of each anther-lobe. Their curvature is thus reduced. The two anther-lobes are joined by the narrow connective, traversed by a vascular bundle (*f*).

If we now examine the cross-section with a higher power, we see most externally the **epidermis** (or **exothecium**), of flat cells filled with violet cell-sap. These epidermal cells are bulged outwards. At the edges of the partition between the pollen-sacs they are rapidly reduced to a small height. Here the separation from the partition takes place. **Stomata** are scattered over the whole surface of the anther, and a small **air-chamber** lies under each of them. To the epidermis succeeds, in the wall of the pollen-sac, a single layer of comparatively high cells, with annular thickenings, the so-called **fibrous-layer** (or **mesothecium**). The rings on these cells are arranged perpendicularly to the surface; they pass over here and there into spiral thickeuings, and anastomose frequently into a network. Passing towards the dorsal side of the anther the walls of the sac become gradually thicker, the fibrous layer being doubled. The remainder of the body of the anther is likewise constructed of fibrous cells. Only the cells which surround the vascular bundle of the connective, and those (*p*) which form the partition between the pollen-sacs, are without thickening ridges.

In order to prepare *surface sections* of the anther, we again select a flower-bud about two-thirds developed. The surface sections show that over the sacs the epidermal cells are longitudinally, the cells of the fibrous layer, on the other hand, are transversely, elongated. Not so on the dorsal surface of the anther, where the fibrous cells appear more isodiametric. Over the sacs the thickening ridges on the outer side of the fibrous cells are weaker, often scarcely recognisable. Often in Angiosperms, as is found also in *Taxus*, the thickening is entirely wanting on the outer surface of the fibrous cells of the wall of the pollen-sac, so that the thickening ridges show U-shaped figures, open towards the exterior. As in drying the cells of the fibrous layer contract strongly, especially in transverse direction, and are hindered in radial contraction by the thickening ridges, so there is brought about an unequal contraction of the fibrous layer, resulting in the opening of the pollen-sacs. (Compare fern sporangia, p. 356). In the pollen-sac of *Pinus*, on the other hand, the epidermis showed the characteristic thickening ridges, as also with the sporangial wall of Lycopodiaceæ; in these cases, therefore, the epidermis plays the mechanical part in dehiscence.

Development of the Pollen and Pollen-Sac.—If we now prepare *cross-sections* through a flower-bud about $\frac{1}{4}$ inch high, we shall find the walls of the sacs consisting, besides the epidermis (Fig.

140, *C*, *e*), of two or three layers of flat (*f*, *c*), and one layer of radially-elongated cells (*t*), the former the **meso-** and **endothecium**, the latter the **tapetal layer**. These last surround the entire sac. The interior is filled with polygonal **pollen mother-cells**, still cohering.—If we next prepare *cross-sections* through a flower-bud about $\frac{2}{5}$ inch in height, we shall see the pollen mother-cells already isolated and in course of division. These pollen mother-cells are recognisable by their white, thick, strongly-refractive wall; their contents are divided into two, or already into four cells, which lie in one (Fig. 140, *D*), or in two planes at right angles (Fig. 140, *E*). **Pollen-grains**, therefore, like spores[1], are produced by quadri-partition inside their mother-cells. The wall of the anther is lined by **tapetal cells**, which are filled with yellow-brown contents. These constitute the innermost layer (*t*) clothing the young sac.—In slightly older flower-buds the walls of the pollen mother-cells are dissolved; the young pollen-grains lie free; the tapetal cells have for the most part lost their individuality, their contents have penetrated between the young pollen-grains. The layer of flattened cells (*f*) underlying the epidermis has strongly developed, and forms the fibrous layer, while the next inner layer is crushed and disorganised. Ultimately, as still older buds show, the unconsumed portion of the tapetel cells, especially in the periphery

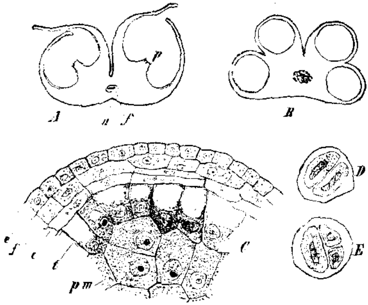


FIG. 140.—*Hemerocallis fulva*. *A*, cross-section through an almost ripe anther, with pollen-sacs opened by cutting; *p*, the partition wall between the sacs; *f*, vascular bundle of the connective; *a*, groove along the connective (× 14). *B*, cross-section through a young anther (× 28). *C*, Part of the previous cross-section of a sac; *e*, epidermis; *f*, the mesothecium, later forming the fibrous layer; *c*, the layer to be displaced (endothecium); *t*, the tapetal layer, to be resorbed later on; *pm*, pollen mother-cells (× 240). *D* and *E*, division of the pollen mother-cells (× 240).

[1] Of Bryophytes and Pteridophytes.

of the sac, takes on an intense yellow-brown colour, a glistening oily appearance, and so forms the oily substance which clings around and upon the pollen-grains.

Substitutes for Hemerocallis.—The species of the genus *Lilium* agree with *Hemerocallis.* The processes of differentiation in the anther take place here, however, later. In flower-buds of the white Lily, *Lilium candidum,* of *L. croceum* and others, four-fifths of an inch high, the pollen mother-cells first begin to divide. In cross-sections through fresh flower-buds the large tapetal cells are very striking from the yellow-brown colour of their contents. The hypodermal cells, as well as all the others which are later on provided with thickening ridges, are densely filled with starch grains.—*Funkia ovata* (May to July) provides likewise a very favourable object for study, and agrees with *Hemerocallis* and *Lilium,* as also do *Agapanthus umbellatus* (Greenhouse, April), species of *Iris,* and many others. *Tulipa* (the Tulips, April, May), and *Hyacinthus orientalis* (the Hyacinth, January to May) are likewise good to use. In *Tulipa* the filament under the anther tapers so sharply that this latter will draw off; in *Hyacinthus* the anthers are almost sessile on the perianth.

Pollen-Grains of Tradescantia.—*Tradescantia virginica* (obtainable from early summer to frost) does not cut so well, but we examine the flowers for their pollen-grains. The stamens of a bud which is ready to expand show us the beautiful sulphur-yellow anthers, fixed upon violet filaments covered with violet hairs (in which we have already studied protoplasmic circulation). The dry pollen-grains are folded (or grooved) on one side (Fig. 141, *A*). In water the fold is levelled out, and the grains become almost ellipsoid; but the side which was furrowed is more strongly bulged.

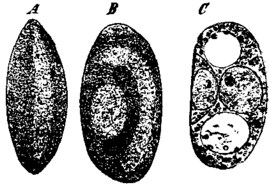


FIG. 141.—*Tradescantia virginica.* *A*, pollen-grain dry. *B*, in water. *C*, young pollen-grain in water, showing the reproductive and vegetative cells (× 540).

Its exine is decorated with fine sinuous lines; the furrowed side also shows this structure, but the exine is there more feebly developed. In the finely granular contents can be distinguished

two brighter homogeneous-looking spots (*B*). These are the two nuclei, of which the one (vegetative) is elliptic, the other (reproductive) is elongated and bent, and almost fills the reproductive cell. The other contents of the pollen-grains are pretty uniformly finely granular. The pollen-grains after some time begin to flatten, whereby the nuclei, together with the contents, are squeezed out. The two nuclei can be seen very beautifully if the pollen-grains are crushed in a drop of acetic iodine-green. The generative nucleus stains more deeply, and in coming out often elongates considerably. If the pollen-grains are placed in the reagents in question, but without crushing, the nuclei show in their natural position inside the grain—the vermiform nucleus always staining very deeply; the elliptic, on the other hand, somewhat more feebly. The rest of the pollen-grain remains at the same time unstained.—If pollen-grains in water have a drop of iodine solution added, we see, after crushing the grain, numerous small blue starch granules in the extruded yellow-brown contents.

If we go back to the younger flowers, and remove the anthers from a bud about ¼ inch long, and crush them in water, we shall see part of the pollen-grains with one nucleus, and part, as in Fig. 141, *C*, with two nuclei lying close together. These two nuclei are, however, separated by a convex partition wall, which encloses one nucleus together with a little protoplasm. This plano-convex cell, in basal outline almost circular, lies always upon the flatter side of the grain, which later on is opposed to the fold, or furrow.—In somewhat older flower-buds we can see that this cell has separated from the wall of the pollen-grain, and lies free in the contents of the grain. It has elongated, and proportionately thinned, and at the same time tapered at both ends; with the exception of the two ends, it is filled by its nucleus. It is this cell which, later on, effects fertilisation, always previously undergoing bipartition in the pollen-tube.—The staining distinction between generative and vegetative nucleus is as a rule much more marked than in *Tradescantia*.

The species of *Leucojum* (Snowflake) agree with *Tradescantia*. The Crown Imperial, *Fritillaria imperialis*, presents another favourable object for investigation. In sections of alcohol material, first placed in water, and then stained with logwood, the [illegible] nuclei in each pollen-grain can be seen very beautifully.

Pollen of Œnothera.—If we open a bud of *Œnothera biennis*

(the Evening Primrose) which is ready to expand, we shall find that the anthers have already dehisced and set free their pollen-grains. These latter are suspended between the anthers by cobweb-like threads. If such threads are stretched out upon an object-slide, they appear under the microscope as exceedingly delicate filaments, some stretched straight, others tangled. The pollen-grains in a dry state are opaque, but their triangular form is at once noticeable. In water, with a higher power, they show as flattened, symmetrically-triangular bodies, with rounded projecting angles. At the base of each of these rounded corners an inner annular thickening of the membrane of the pollen-grain is to be seen. The contents of the pollen-grain appear finely granular; the two nuclei are only recognisable in the contents of the ripe grain with extreme difficulty. In sulphuric acid the exine assumes a red-brown colour. By it an outer, thin, yellow-coloured layer is lifted up, forming folds upon an inner, thicker, red-brown layer. Both layers coalesce in the uncoloured wall of the rounded corners. From the side walls of the corners fine teeth project inwards. The apex of the corners is dissolved by the sulphuric acid. The fine filaments, binding the pollen-grains together, resist water, sulphuric acid and potash, and are insoluble also in alcohol. If the grains are treated with 25 per cent. chromic acid, their membrane is soon dissolved, and in all cases the strongly-cuticularised portion somewhat sooner than that which is either not, or but slightly cuticularised, which remains for a time as colourless swollen caps covering the rounded angles. Later on, these also are dissolved; and ultimately even the cobweb threads between the grains.

Development of the Pollen-Tube.—From the stigma of an older flower of *Œnothera* pollen-grains can be lifted off which have already germinated and developed **pollen-tubes.** In this case also there is an inner colourless intine surrounding the contents of the pollen-grain, which develops into the pollen-tube through one or other of the angles. The formation of a pollen-tube takes place usually at one angle only, that one which happens to be most favourably placed; or if more than one tube is started, only one continues to develop. Instead of *Œnothera*, an *Epilobium* (Willow-herb), or a *Fuchsia* can be used for study.

Longitudinal sections of the arms of the style of flowers of *Œnothera*, etc., which are past their prime, will show the pollen-tubes, developed from the corners of the pollen-grains, traversing

the tissue of the style. With magenta the contents of the pollen-grains and tubes will commonly stain deeply, the tissue of the style only very slightly; and hence the pollen-tubes can be traced with great ease, and often in large numbers. Similarly iodine can be used, and will demonstrate the usual presence of considerable quantities of small starch grains in the stylar tissue which the pollen-tubes are traversing.

Later in this chapter we will consider the question of the artificial culture of pollen-grains.

Pollen of Malvaceæ.—Still a few other pollen-grains of specially characteristic form may be considered. The Malvaceæ are distinguished by remarkably large pollen-grains; we examine those of the Hollyhock, *Althæa rosea*. In water these appear globular, opaque, studded with colourless spines. They become very beautifully transparent in carbolic acid and in chloral hydrate, much less so in oil of cloves, still less in oil of lemon, all of them media which are used in such cases to make objects transparent. The preparations are best in carbolic acid, so that we will use this. Their surface view shows us that the colourless exine is studded, at approximately equal distances, with large pointed spines. Between these are scattered others, short, blunt, of variable thickness.[1] Regularly-distributed circular openings, appearing rose-coloured, traverse the exine, the basal surface of which is finely punctate. The contents of the pollen-grain appear uniformly finely granular, the nuclei are very difficult to distinguish. The optical section of the grain shows us clearly the form of the large and small spines, and of the canals penetrating the exine. An exceedingly delicate intine can be traced as a boundary to the contents; it bulges a little, papilla-like, into the exit-pores of the exine. In concentrated sulphuric acid the exine is quickly stained red-brown, and its structure is then very clearly shown.

The pollen-grains of most other Malvaceæ resemble those of *Althæa*. In *Malva crispa*, a hardy annual species not infrequently cultivated, for example, the pollen-grains are shaped just as in *Althæa*, excepting that the spines on the membrane are all alike; between the spines lay scattered the exit-pores or canals; the membrane appears besides finely punctate.

[1] A third form can be occasionally found in which a short spine is situated upon a hemispherical, dome-like base.—[Ed.]

We can use the large pollen-grains of *Althæa rosea*, or of *Malva crispa*, for the purpose of preparing sections. Material hardened in alcohol is best for our purpose, and we lay it before use in mixture of equal parts alcohol and glycerine. We prepare a thick solution of gum, to which we add a small quantity of glycerine, place a drop of this solution upon the smooth end of a piece of elder-pith, and place some pollen-grains on the drop. These are stirred into the gum, and then the drop either allowed to dry in the air, the elder-pith being placed in a per-

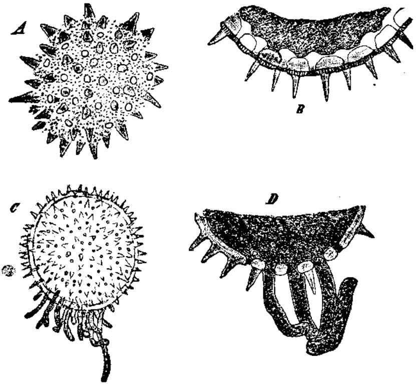


FIG. 142.—Pollen-grains of *Malva crispa*. *A*, piece of a grain seen from the surface. *B*, portion of a section through a grain. *C*, a pollen-grain removed from the stigma, and showing pollen-tubes. *D*, portion of a similar grain in optical section (*A*, *B* and *D*, × 540, *C*, × 240).

pendicular position, or else is hardened by laying for several hours in alcohol. After this has happened, we prepare delicate sections through the gum with a very sharp razor. The sections can be small to any degree, but must be exceedingly thin. They are laid in water or in dilute glycerine, whereby the gum is dissolved and the enclosed sections of pollen-grains are set free. In such sections the structure of the wall of the pollen-grains can be studied in all its details. Such a section of *Malva crispa* (Fig. 142, *B*), shows the exine to consist most externally of

a delicate outer layer beset with spines; next a delicate "rodlet layer," which corresponds with the dots seen in surface view (Fig. 142, *A*); and a thick homogeneous inner layer, forming convex projections inwards. The intine is swollen under the exit-pores, but elsewhere forms a delicate membrane. If such a section is treated with chlorzinc-iodine, the outer layer of the exine and the spines scarcely stains at all, the thickening layer of the exine stains yellow-brown, and the intine blue. The intine in this case is relatively rich in cellulose. The contents of the pollen-grains swell and colour violet, owing to the presence of starch grains, which swell and stain. The unstained as well as the stained sections, provided they are extremely thin and strongly magnified, show us that the exit-pores of the exine are closed externally by a very delicate membrane which passes over them.—In order to obtain the best possible sections of pollen-grains, capable of use for the highest microscopical purposes, and of staining for cellulose and pectin, it is necessary to embed the material in paraffin, and cut with a microtome, after the method described in Chapter XXXII.

If we examine the stigma of an older flower of *Malva crispa* under the simple microscope, or with a strong lens, many pollen-grains will be found upon it. On the side turned towards the stigma these will have produced numerous pollen-tubes. If such a grain, the pollen-tubes of which are still short, be removed and examined, we can readily determine that the pollen-tubes come from the canals or exit-pores of the exine (Fig. 142, *C*). This is shown still more beautifully in optical section, after the grain has been made transparent in carbolic acid (Fig. 142, *D*).

Pollen of Cucurbita.—The large pollen-grains of species of *Cucurbita* have always been specially noticeable for the valves which close the places of egress in the exine (Fig. 143, *A*). In water, yellow oil-drops come off from the surface of the exine, the grains soon evacuate their contents, and the structure of the membrane then becomes clear. The exine is studded with regularly-distributed large spines, and between them very numerous minute ones. The exit-places are round, the valve is lifted up, either on one side or altogether, by the papilla-like bulging of the intine. The valve has the structure of the surrounding exine, and bears one or more of the large spines.—Very good figures are obtained in oil of lemon, less useful in oil of cloves. On the other hand, the figures in chloral hydrate are to be preferred to those in

carbolic acid. In a word, *the most favourable clearing medium for each object must be found by experiment.* Upon the preparation in oil of lemon or in chloral hydrate we can determine, by optical sections, the position of the valve inside the exine, in which it is found wedged with its base somewhat broadening inwards (Fig. 143, *B*). Under the valve can be seen the bulging of the intine. In sulphuric acid the oil-drops on the exine become blue, and the exine itself slowly becomes brown. The valve is thrust off by the swelling contents. In 25 per cent. chromic acid the entire pollen-membrane is soon dissolved; the intine resists somewhat longer, and, at the moment when the exine disappears, can be traced as a strongly swollen, homogeneous membrane. The pollen-grain has previously emptied itself, whereby the observation of the intine is considerably facilitated. In sulphuric acid, on the other hand, the intine is immediately dissolved, the exine remains, the extruded contents of the pollen-grain are gradually, as in other cases, coloured rose-red.

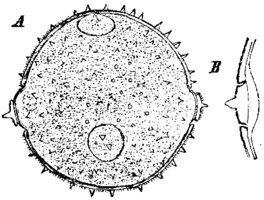


FIG. 143.—*A*, entire pollen-grain of *Cucurbita Pepo*, seen in surface view, and partly also in optical section; taken from a preparation in oil of lemon (× 240). *B*, pollen-grain of *Cucurbita verrucosa*; fragment of a cross-section through the membrane (× 540).

Compound Pollen-Grains.—Of compound pollen-grains, which occur alike in Monocotyledons and Dicotyledons, we will select those of the Ling, *Calluna vulgaris*. The grains here are joined in fours, and usually grouped tetrahedrally. The pollen-membrane shows only slight protuberances, and usually three exit-places for each grain. The various species of *Erica*, *Azalea* and *Rhododendron* agree in all essentials with *Calluna*.—In species of *Acacia*, as in the Mimoseæ generally, the pollen-grains form groups of four, eight, twelve and sixteen, and even more cells, but can occur also separately.

Artificial Culture of Pollen-Grains.—The artificial production of pollen-tubes is as a rule very easy. In a from 3 to 30 per cent. solution of sugar, in spring (not distilled) water, to which 1·5 per cent. gelatine has been added, most pollen-grains readily put out their tubes, in which protoplasmic movement is beautifully

seen. The formation of tubes takes place quite certainly and rapidly in 5 per cent. solution of sugar and 1·5 per cent. gelatine from the pollen-grains of *Pæonia*, *Staphylea*, and even from *Tradescantia* when the pollen-grains are taken from freshly-opened flowers. The most favourable objects are perhaps species of *Lathyrus*, *e.g.*, Sweet Pea, Everlasting Pea, etc., in 15 per cent. solution of sugar and 1·5 per cent. gelatine. This solution must be freshly prepared; the sowing is best performed in a suspended drop in a moist chamber (see p. 279).

Though many pollen-grains have a wide range in this respect, for most the degree of concentration of the sugar solution is of vital importance, and ought therefore to be experimentally determined for each species. A few further cases may be given. Most species of *Allium* (onion) produce their pollen-tubes in a 3 per cent. solution of sugar, and show active protoplasmic streaming. *Tulipa Gesneriana* requires from 1 to 3 per cent. sugar; pollen-grains of *Leucojum æstivum* (snow-flake) germinate very easily and rapidly in 3 to 5 per cent., and the same with *Narcissus pœticus* (pheasant-eye narcissus); *Convallaria majalis* (lily of the valley) in 5 to 20 per cent.; *Iris sibirica* in from 30 to 40 per cent. The pollinia of orchids produce pollen-tubes in from 5 to 10 per cent. sugar, usually after from twenty to forty hours. Amongst Dicotyledons the pollen-grains of *Torenia asiatica* germinate very readily, producing pollen-tubes in 10 per cent. sugar solution after about two hours, and the tube grows so rapidly that, with a high power, its tip can be seen to move across the field of view. Species of *Gloxinia* behave in much the same way in 10 per cent. solution; *Papaver* (poppy), 5 per cent.; species of *Sedum*, and *Viola tricolor* (pansy), 30 per cent. In all these cases 1·5 per cent. of gelatine is added.—The pollen-grains invert the cane-sugar of the culture-fluid, the invertin being present in the grain prior to germination. The pollen-grains likewise contain diastase, and are capable therefore of dissolving starch-paste if present in the culture-fluid, as, *e.g.*, 1 per cent. potato starch-paste, made at boiling temperature. This occurs whether they have germinated or not, and even if they have burst in the culture-fluid.

Most pollen-grains burst if their cultivation is attempted in water only. Some, however, produce their pollen-tubes in this medium without bursting, *e.g.*, those of *Nicotiana* (tobacco), *Galeobdolon luteum* (= *Lamium Galeobdolon*, the yellow dead-nettle),

Lysimachia nummularia (Creeping Jenny), *Agapanthus*, *Lobelia*, *Lilium*, *Aquilegia*. Many pollen-grains lose their capacity for germination if the water contains small quantities of mineral salts, since these are poisonous to them. Sometimes transverse walls are formed in the pollen-tube, as, *e.g.*, in *Montbretia* (*Tritonia*), cultivated in 7½ per cent. sugar and 1½ per cent. gelatine. Separate protoplasmic movements may then take place in each cell. The growth of the pollen-tube in this case also is exceedingly rapid, the elongation being visible with such a low magnifying power as 200 diameters.

CHAPTER XXIX.

THE GYNŒCIUM OF ANGIOSPERMS.

MATERIALS USED.

Delphinium Ajacis, just fading; fresh, or in alcohol. Or *Helleborus*, or other similar Ranunculaceous plant.

The same of some Liliaceous plant, *e.g.*, Tulip, Hyacinth, *Lilium*, *Hemerocallis*.

The same of a *Primula*, or a *Lysimachia*.

The same of an *Œnothera*, or *Epilobium*.

Full blown flowers of *Aconitum*.

The same of *Monotropa*; preferably fresh. Or of some species of *Pyrola*, or *Orchis*, or *Gloxinia*.

Orchis species, with swelling ovary, one or two weeks after pollination; fresh; or flowers of *Gloxinia*.

Torenia asiatica, unpollinised flowers, and those pollinised thirty-six hours before; fresh.

REAGENTS USED.

Potash—Celloidin—Logwood—3 per cent. sugar solution—2 per cent. acetic acid.

Structure of Monocarpellary Pistil.—Let us first obtain a general idea of the structure of the **Ovary.** For this purpose one of the Ranunculaceæ is very well suited, *e.g.*, *Delphinium Ajacis*, the Larkspur of gardens. We choose an old flower, from which the petals and stamens can be easily removed, and observe three **pistils** (or **carpels**) left standing in the centre. Even with superficial observation we can distinguish upon the pistil the lower, green, swollen portion—the **ovary**, and the thin part, here rose-coloured, into which the ovary narrows above—the **style.** This latter ends with the **stigma,** which in this case is not specially delimited.—We now prepare *cross-sections* through all three ovaries together, and examine them with a low power, or with the addition of a little potash. The cross-section (Fig. 144)

shows us a single **cell** or **loculus** in each ovary. Apparently it is a single reproductive, or **carpellary leaf**, which forms each such ovary. We can conceive the carpellary leaf as having folded inwards and its edges grown together. To such an origin points, moreover, the **ventral suture**, which we find in the median plane of the ovary on its side turned towards the centre of the flower. Such an ovary, composed of one fertile carpellary leaf, is **monocarpellary**; when a number of such monocarpellary ovaries are found in a single flower, as is the case in our example, the flower is said to be **apocarpous.** The ovaries are here free to their base, and inserted upon the summit of the **floral axis**, *i.e.*, they are **superior**, or **free.** The entire female sexual apparatus of the flower may consist of one or of many pistils, and is designated the **gynœcium.** Our cross-section shows clearly the groove on the ventral side; and with stronger magnification we can here follow the outer epidermis through the entire thickness of the wall, and see it continue into the epidermis of the ovarian cavity. It is interesting that this inner epidermis also possess **stomata.** The wall of the ovary is traversed by a number of vascular bundles, of which most appear on the dorsal side, and some near the edges of the carpel on the ventral side. The edges of the carpellary leaf are somewhat thickened, and form, on the cavity side, the **placentæ** (*p*). From these the **ovules** (*s*), in agreement with the number of placentæ, arise in two rows. With the ovules we shall concern ourselves later on, and for this purpose we put our preparation on one side.

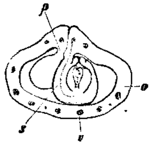


FIG. 144.—*Delphinium Ajacis.* Cross-section through an ovary. *o*, wall of the ovary; *v*, vascular bundles in the wall; *p*, placenta; *s*, rudimentary seed or ovule (× 18).

Instead of *Delphinium*, and available so early in the year as February, we can use the pistils of the stinking Hellebore, *Helleborus fœtidus.* They agree in all essentials with the above. Sections taken through flower-buds at various stages will show moreover the method of union of the edges of the carpellary leaf. Some of the sections will show these edges unjoined but in contact; in others, the epidermal cells of one thickened edge will be seen to grow out, papillately, between the similar cells of the other margin, so as to "dovetail" the edges together. Sections of the same young flower-buds may show the division

of the pollen mother-cells into tetrads. For earlier examination still, the Christmas Rose, *H. niger*, may be used.

Tricarpellary Pistil.—The ovary of Liliaceæ is free, trilocular; we select at convenience the Tulip, Hyacinth, a Lily, or *Hemerocallis* for examination. In the Tulip the three stigmatic lobes are sessile upon the ovary, without style. In the Hyacinth the style is short, the stigma small, slightly trifid. In *Lilium* the style is long, the stigma tripartite. In *Hemerocallis* the style is very long, the stigma likewise tripartite, but very small. *Cross-sections* show us a **trilocular** ovary, composed of three closed carpellary leaves which have grown together. Here, neither outside nor in, is a limit between the tissues of the individual carpellary leaves to be recognised; and a single continuous epidermis covers the exterior of the whole structure. In *Yucca*, another genus of the Liliaceæ, the limits of the three carpellary leaves are marked externally by grooves, and internally by narrow radial pear-shaped cavities in the tissue—each cavity being lined by a distinct epidermis. Three carpellary leaves therefore form in these cases a **syncarpous, trilocular ovary.** Each of the three carpellary leaves combined into this trilocular ovary bears, corresponding to its two edges, two rows of ovules; *i.e.*, the placentæ lie here in the inner angles of the loculi or cells of the ovary. The placentation is therefore marginal, as in *Delphinium;* but as they arise from the angles of the cells, and therefore in the centre, it is specially designated **axile placentation.**

Structure of the Style and Stigma.—*Cross-sections* through the style of *Hemerocallis* show us in it a central triangular passage—the **pollen-passage.** Three vascular bundles are distributed at the three angles of the pollen-passage. A *longitudinal section* through the apex of the style, and therefore through the stigma also, shows us the surface of this latter grown out into long papillæ. This phenomenon is very general upon stigmatic surfaces; *Hemerocallis*, however, offers still another interesting condition, in that the cuticle of the papillæ is raised up by the formation of slime or mucus. This cuticle is spirally striate, and, in accordance with this, its upheaval follows a spiral line. At length the cuticle is entirely loosened from the inner layer of the membrane, and is exfoliated from the papillæ.—Other Liliaceæ, etc., likewise show a hollow style; in most cases, on the other hand, the style is solid, but filled either with cells easily

passing out of lateral union, or else provided with swollen side walls, between which the pollen-tubes can easily grow downwards. In either case a valuable conducting tissue for the pollen-tubes is provided (compare p. 387).

Pistil of Primula.—Another free or superior ovary exists in the flowers of the species of *Primula* (Primrose, Auricula, Cowslip, etc.). These are **dimorphic**, *i.e.*, have short-styled and long-styled ovaries, and stamens inserted high up or low down upon the tube of the corolla. A *median longitudinal section* through the ovary shows us that the floral axis is prolonged into the cavity of the ovary, and here enlarges into a mushroom-like swelling. On grounds of comparative morphology, however, it is believed that this central structure is a part of the carpellary leaves which adheres to the floral axis. At its apex this swelling projects, papilla-like, into the pollen-passage of the style. The entire surface of this swelling is covered with ovules. We have here a **free central placenta.** The wall of the ovary is in no way directly connected with this placenta. We can be quite convinced of this by cross-sections in which the wall of the ovary appears as a free ring around the central placenta, or, if an equatorial incision is made round the ovary, the style and upper part of the ovary can be lifted off like a cap from the mass of ovules, and the prolongation of this central swelling will be withdrawn from the pollen-canal. Wanting also in the wall of the ovary are the points of separation which enable us to determine the number of carpellary leaves concerned in its formation; these, however, are assumed to be five, from consideration of the numerical symmetry of the other floral parts, and from the circumstance that in many Primulaceæ the fruit-capsule dehisces at its apex with five teeth. In *Primula* itself the number of the teeth with which the capsule opens is irregular. Instead of *Primula*, species of *Lysimachia* (Loose-strife, Creeping Jenny, Money-wort, etc.), or of *Anagallis* (Scarlet Pimpernel, Bog Pimpernel, etc.), can be used with the same results; they all bear their ovules on a free central placenta.

Adherent Ovary of Œnothera.—A multilocular inferior or **adherent ovary** we will find in *Œnothera biennis* (evening primrose), or any other *Œnothera*, or in *Epilobium*. The ovary here often lies considerably below the point of insertion of the floral envelopes. The *cross-section* shows four cells or loculi. The placentæ arise from the inner angles of the cells; they

project somewhat into the cavity of the cells, and bear two or three rows of ovules. The middle line of each cell is represented by a slight depression. At these points lie weak vascular bundles while a strong one externally and a weaker one internally lie at the outer edge of the septa. The inner one, by means of horizontal branches, which the cross-section often lays bare, is connected with the bundles which occupy the central tissue between the four cavities. These for their part nourish the placentæ. The wall of the ovary contains numerous raphides which, set free from their cells, lie scattered over the whole section.

An adherent or "inferior" ovary is, from a philogenetic or evolutionary point of view, to be considered as a hollowed floral axis, filled up by and completely adherent to, the ovary of a syncarpous pistil. Its wall might be expected, therefore, to sometimes, at least, show signs of its composite character, and possibly the two bundles juxtaposed to the septa are such. The anatomical and physiological data for this theoretical view are, however, often wanting, though *Eschscholtzia californica* (the yellow Californian poppy, an annual very common in gardens), many Rosaceæ, Saxifragaceæ, etc., are of great interest in this connection. Often we are bound to conclude, however, that the structure of the wall of an "inferior" ovary, *i.e.*, of an ovary wall plus receptacular tube does not recognisably differ from that of a free ovary, *i.e.*, of an ovary wall alone.

Structure of the Ovule of Aconitum.—We will now endeavour to become acquainted with the structure of the ovule, and at the same time learn something of the processes of fertilisation in Angiosperms. To study the separate parts of the ovule, we first prepare *cross-sections* through the ovary of the Monkshood, *Aconitum Napellus*, or of some other species of *Aconitum*. We select a flower in full bloom, strip off the other parts of the flower, and then cut through the three ovaries together. Care should be taken that the sections are made correctly, at right angles with the long axis of the individual ovaries. The number of sections must be considerable in order with more certainty to cut an ovule correctly. We glance over the sections, and select those which appear likely. In case the section is not delicate enough, we can help matters with a little potash. If an ovule is cut centrally, it appears as in the adjoining Fig. 145. The ovary is monocarpellary, in all essentials resembling that of *Delphinium*; the ovule arises from a marginal placenta. It is inserted thereon with a

stalk, the **funicle** (f); the free part of this is very short, the rest of it has grown to the side of the ovule, forming upon it the **raphe** (r). In the body of the ovule we note first of all the inner conical mass of tissue, the **nucellus** (n). This corresponds with the **macrosporangium** of Vascular Cryptogams. The nucellus is encased in two **integuments**, an inner (ii) and an outer (ie). The inner is developed on all sides right to the base of the nucellus, the outer is wanting on the side of the raphe, since it joins on both sides to the funicle. The inner integument leaves free a narrow canal between its upper edges, extending to the nucellus; this canal is known as the **micropyle**. The funicle is traversed by a vascular bundle, arising from the placenta, which in many, but not all, cases can be traced to the base of the nucellus. The tissue adjoining the base of the nucellus, here distinguished by its brighter colouring, is known as the **chalaza** (ch). In the long axis of the nucellus is noticeable a large cell, forming quite a cavity; this is the **embryo-sac** (e). At its base can be seen some globular cells, which in *Aconitum* (and Ranunculaceæ generally) are very strongly developed—the **antipodal cells** (a).

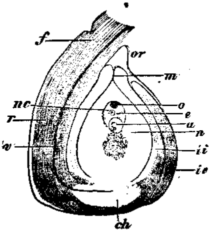


FIG. 145. — *Aconitum Napellus*; median longitudinal section of an ovule. *f*, funiculus; *r*, raphe; *v*, vascular bundle of the funicle; *ie.*, outer integument; *ii*, inner integument; *n*, nucellus; *ch*, chalaza; *e*, embryo-sac; *a*, antipodal cells; *o*, the oosphere; *nc*, nucleus of the embryo-sac; *m*, micropyle; *or*, wall of the ovary (× 53).

In specially favourable cases we can determine that they are three in number. In the apex of the embryo-sac we can also see a small cell, which, however, is only recognisable in perfectly median sections; it is the **oosphere**, sometimes called the embryonic vesicle or germinal vesicle (o). The ovule as a whole is described as **anatropous**, *i.e.*, turned back, because the body of the ovule does not lie in direct continuation of the funicle, but appears laid by the side of it, with one side grown to it, and the micropyle turned to the base of the funicle. This form of ovule is by far the most common in Angiosperms.—If we now compare our preparation of *Delphinium* (Fig. 144) with that of *Aconitum* (Fig. 145) we shall see that the structure of the ovary and ovule in the two cases is quite identical; the distinction only is that in *Delphinium*, as very

commonly in Ranunculaceæ, the two integuments of the ovule are blended together.

Embedding in Glycerine-jelly, and in Celloidin.—In order to prepare sections of the ovule of *Aconitum*, we can separate one from the ovary, and cut it between the thumb and forefinger, in the method already known to us. If the ovule is correctly arranged between the fingers, we shall in this way more rapidly obtain true median sections. In this and in other like cases the ovule may, with advantage, be first embedded in glycerine-jelly or in celloidin (celluloidin), and then cut. The glycerine-jelly must be tolerably firm, *i.e.*, must contain a comparatively large proportion of gelatine. In celloidin only alcohol material can be embedded. We pour the solution of celloidin [1] into a small box made of writing-paper, and immerse the ovule in it. The celloidin is then allowed to stand in the air till it has acquired a firm skin, when it is laid in 82 per cent. alcohol. Here after some hours, it acquires the consistence of cartilage, remaining transparent. Celloidin and object are cut together, and the sections laid in glycerine or glycerine-jelly, without its being necessary to remove the celloidin. The sections can be stained with carmine or with logwood (hæmatoxylin), but not with aniline colours, as these latter colour the celloidin as well. If the celloidin has been procured in cakes, it must be dissolved before use in equal parts of ether and absolute alcohol. In order to make ovules which are to be embedded in glycerine-jelly or in celloidin still more visible, they can be previously stained with watery logwood; the ovules must then, however, after previous washing in water, be again dehydrated in absolute alcohol, before being placed in the celloidin. Objects which, in order to make them available for section-cutting, must be permeated with celloidin, are first treated with dilute solution of celloidin, in which the object must lie often for days before it is transferred to, and embedded in, the thick celloidin solution. Other methods of embedding will be found described in Chapter XXXII.

Embryo-Sac of Monotropa.—We will now take in hand the study of the interior of the **embryo-sac.** The most favourable object for this is *Monotropa Hypopitys*, the Bird's-nest Rape,

[1] To be obtained of Dr. Grübler, in Leipzig, Dufour-strasse, 17. In cakes at about 8s. each, or in solution at 11s. the kilogramme. Also of Messrs. Southall Bros. & Barclay, manufacturing chemists, Birmingham.—[Ed.]

a pale yellowish parasite, found chiefly under beech and fir trees. This plant is so favourable for the otherwise difficult investigation of the embryo-sac that no pains should be spared, if possible, to obtain it.[1] It flowers in July and August, and mnst be examined fresh, as in alcohol it becomes brown and opaque. The plant bears carriage very well, and can be preserved healthy for a very long time in a glass of water. Supplies may in this way be obtained from a distance.

If it is necessary to keep *Monotropa* in alcohol, this latter should have sulphur dioxide added to it, since in pure alcohol the object becomes dark brown. For this purpose sulphur dioxide gas should be passed into the alcohol. This operation will take about a minute if we pour a little 80 per cent. sulphuric acid over sodium sulphate in a suitable vessel, and conduct the gas evolved into the alcohol. To each 100 c.c. alcohol about $\frac{1}{2}$ gram sodium sulphate should be allowed. The object remains in this sulphurated alcohol for about twenty-four hours, and can then be transferred to pure alcohol.—With *Monotropa* agree the various species of *Pyrola*, or "winter-green," excepting that their ovules are smaller. About a dozen species of *Pyrola*, all hardy herbaceous perennials, can be readily enough cultivated in gardens, either from seeds or division of the roots, selecting for the purpose a shady border, with a sandy peat soil.

The *cross-section* through the under part of the ovary shows this to be four-celled, five-celled in *Pyrola*. The placentæ are strongly swollen, and bear on their surface very numerous, slender, closely packed ovules. The two halves of the placenta in each cell are separated to some little distance by a radial groove. In the upper part of the ovary these grooves extend to the centre of the ovary, and there coalesce. We see then four strong pairs of placentæ, each placed near the centre of a septum, and which appertain to two adjoining loculi; the pairs are easily separated from one another with the needles. We obtain ovules for study by removing a portion of the wall of the ovary with the forceps, and stripping off with needles the ovules from the placenta thus exposed. We place them in pure water, or in 3 per cent. solution of sugar, in which the ovules remain longer unchanged. If we take our material from an oldish flower, in which the stamens

[1] It is hardly likely to be regularly obtainable in England, so that the alternative plants referred to must be relied on for material.—[Ed.]

have already dehisced, we shall find the ovules in part fully developed and not yet fertilised, in part already fertilised. Between the ovules we come often upon fragments of pollen-tubes.

The receptive ovule has the appearance of the adjoining Fig. 146. It is transparent, and can be focussed for optical section.

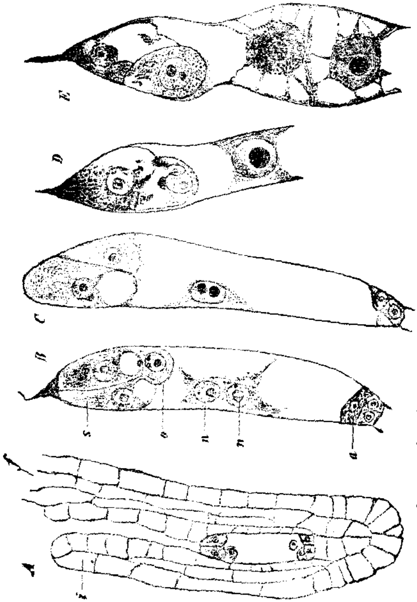


Fig. 146.—*Monotropa Hypopitys.* *A*, an entire ovule, in which *f* is the funiculus; *i*, the integument (× 240). *B* and *C*, the entire embryo-sac, in which are *s*, the synergidæ; *o*, the oosphere; *n*, the nucleus of the embryo-sac; *a*, the antipodal cells; *D* and *E*, upper parts of the embryo-sac; in *E*, the first division for the formation of endosperm (B to E × 600.)

We recognise it as an **anatropous ovule**, with but one integument (*i*). The whole interior of the ovule is filled by the **embryo-sac**; we miss the nucellus, which, during development, is pressed back by the embryo-sac. The apex of the embryo-sac is occupied, as we can clearly see, by three cells. These three cells form the **egg-apparatus or germinal apparatus.** They are not of equal

value; the two upper are the assisting-cells, or **synergidæ** (Fig. 146, *B*); that more deeply inserted is the **oosphere** (*o*) (germinal vesicle or embryonic vesicle). The synergidæ, as can be easily seen, have in their lower part a vacuole, are filled above with protoplasm, and here contain the **nucleus.** The oosphere, on the other hand, has its cavity above, and the main mass of its cell-protoplasm, with the nucleus, below. Both synergidæ are not always visible, as one can cover the other (Fig. 146, *C*). At the base of the embryo-sac the **antipodal cells** can usually be recognised without difficulty, and three can usually be counted. In the interior of the embryo-sac is usually found a **nucleus,** with a **nucleolus** (Fig. 146, *A*); but in other cases there are two nuclei (*B*) or a nucleus with two nucleoli (*C*); and we judge from this that the one nucleus which we always ultimately find arises from the union of two.—Ovules, the fertilisation of which has already commenced, can be recognised by the changes which the synergidæ have undergone. These appear strongly refractive, both or only one being thus modified. It is then certain that a pollen-tube has penetrated to the embryo-sac, and while it is not easy to see it within the micropyle, it is still not difficult to recognise, projecting beyond the micropyle, the piece torn off from it in preparation. The apex of the pollen-tube, however, has penetrated to the synergidæ, and a reproductive cell of the pollen-tube has passed from it to the oosphere. With careful examination we may happen, in oospheres which border on synergidæ that are thus changed, to find two nuclei (*D*), one larger, the original nucleus of the oosphere, the **oo-nucleus,** and close by it also a smaller, the **spermo-nucleus,** which has penetrated from the pollen-tube. This latter quickly increases in size. We can find stages of conjugation between the oonucleus and this spermo-nucleus, afterwards see only one **embryo-nucleus,** with two unequal nucleoli, of which the smaller arose from the spermo-nucleus (*E*), and ultimately an embryo-nucleus with only one nucleolus[1]. While the oosphere is being fertilised, the highly refractive masses of substance in one or both synergidæ diminish; they are apparently used for the nourishment of the **oospore.**

At the same time with these changes in the egg-apparatus the formation of **endosperm** has commenced in the cavity of the embryo-sac; *i.e.*, we see the embryo-sac divided by walls. The endosperm-formation here, therefore, takes place by **cell-division**; while in other equally frequent, even more frequent, cases, the

[1] See note on p. 408.

nucleus of the embryo-sac and its descendants at first divide free, and only at a later stage of development the formation of partition walls between these nuclei commences. The process, as seen here, takes place in general in such embryo-sacs as show slow, and on the whole inconsiderable, increase in size. Where, on the other hand, the embryo-sac increases very rapidly in size after the fertilisation of the oosphere, there nuclear division without cell-division first takes place, and cell-formation, *i.e.*, the formation of the partition walls, first begins when the embryo-sac is approximately fully developed.—As a result of fertilisation the oospore has acquired a delicate cellulose membrane, and soon begins to elongate into a sac, and after some time penetrates with its apex into the body of the endosperm, where the apex of the sac produces a few celled embryo.—We have thus far examined these ovules only in pure water or in sugar solution; if we wish to see the nuclei show up clearly, we must treat the ovules with 2 per cent. acetic acid. In this way we obtain very sharply-defined figures in most ovules, and at the same time fix dividing phases of the nuclei, although into these processes we do not propose at present to go more deeply. Staining media cannot be recommended, since they stain also the nuclei in the integument, and in this way disturb the view into the interior.

Embryo-Sac of Orchideæ.—Instead of *Monotropa* various Orchids (*Orchis* and other genera) can be used for study. Fertilisation takes place in these a good while after pollination, from three to ten days, according to species, and in ovaries which are already greatly enlarged. These are cut open, ovules removed with the needles from a placenta, and transferred to water or 3 per cent. solution of sugar. We can at once recognise the structure of the fully-formed ovule (Fig. 147), which is very like to that of *Monotropa;* but there are two integuments, as commonly in Monocotyledons, and an air-cavity in the neighbourhood of the chalaza. This air-cavity makes observation more difficult, for it is filled with air, which also penetrates between the integuments. The ovule, in water or in 3 per cent. sugar solution, must therefore be freed from air under the air pump. Often even a slight pressure upon the cover-glass serves to remove the most disturbing air, found between the integuments. The nucellus in Orchideæ also is quite displaced by the embryo-sac; as a relic of the nucellus a strongly refractive cap of substance is often still to be seen at the apex of the embryo-sac. The egg-apparatus (*os*)

is constructed as in *Monotropa*, except that the oosphere is less deeply inserted. The antipodal cells are not visible; in their place is a strongly-refractive substance, in which lie, in fact, three nuclei, recognisable, however, with great difficulty. The pollen-tube can, more easily than in Monotropa, be traced to the synergidæ; the changes which the synergidæ undergo are the same. Two nuclei, moreover, are again found in the fertilised oosphere. Endosperm is in general not formed in the Orchideæ.

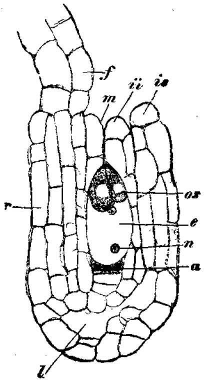


FIG. 147.—*Orchis pallens.* Receptive ovule. *os*, egg-apparatus; *ii*, inner, *ie*, outer integument; *l*, air-cavity. The other letters are as in the previous figures (× 240).

In default of *Monotropa* and of Orchidaceæ transparent ovules for investigation are provided by various Gesneraceæ, and, above all, the large-flowered *Gloxinia hybrida* of gardens. The ovule, having only one integument, is so far transparent that the egg-apparatus is clearly visible. It shows the two synergidæ, and the, in this case flask-shaped, oosphere. In some cases two oospheres can be present. The embryo-sac in its upper part is swollen, but narrows suddenly below; the antipodal cells in the lower end are not distinguishable with certainty.—It is comparatively easy to obtain an insight into the structure of the egg-apparatus by means of sections through the ovary of species of *Lilium* or *Narcissus*. As the ovules in the ovary cells of these plants are arranged quite radially, longitudinal sections of ovules can be obtained by cutting *cross-sections* of the ovary. The sections should be cut from fresh ovaries, and examined in 5 per cent. sugar solution, and a considerable number should be examined in order to obtain the required structure with certainty.

Embryo-Sac and Fertilisation in Torenia.—One of the most favourable plants for the study of fertilisation is, however, the Scrophularineous plant *Torenia asiatica*. This stove-evergreen from the East Indies is now cultivated very generally in gardens,

and bears flowers the whole year round. It is remarkable in that its embryo-sac grows upwards into the micropyle, and hence the whole egg-apparatus appears without any other covering than the wall of the embryo-sac. *Cross-sections* through the free, elongated ovary show this to be two-celled; the two axile placentæ project as pads into the loculi. They are covered with numerous ovules.—For the purpose of their study we remove a part of the wall of the ovary, and strip off the ovules from the placenta, preferably under the simple microscope. We observe them with advantage in a 3 per cent. solution of sugar. The ovules are anatropous, or, more correctly, somewhat campylotropous, for the embryo-sac and the integument are bent in their upper part (Fig. 148, *A*). The free part of the funicle (*f*) of the ovule is pretty long; only one thick integument is present. The embryo-sac (*e*) projects with its upper end out of the micropyle. This protruding part is swollen and pointed at its apex; it lies against the funicle. It is difficult to follow the embryo-sac in the interior of the ovule, but by running in a little potash we can, at the beginning of its action, convince ourselves that it immediately impinges on the ovular integument, is first very narrow, then swells somewhat spindle-shaped, and (*e**) again narrows at the base. Our preparations in sugar solution show, in the free apex of the embryo-sac, the two synergidæ and the oosphere; once more therefore, as always, three cells form the egg-apparatus. According to the position of the preparation two synergidæ are to be seen (Fig. 148, *B*), or one conceals the other (*C*). At the apex of each synergida we notice a homogeneous, strongly-refractive cap, sharply defined against the finely granular portion behind; this is the so-called **filiform apparatus.** If such a preparation is treated with chlorzinc iodine, the caps of the synergidæ are seen to colour blue. They consist therefore of cellulose. The rest of the substance of the synergidæ and of the oosphere colours yellow-brown. Careful examination shows that the membrane of the embryo-sac is open over the caps of the synergidæ (*B*, *C*). The filiform apparatus therefore forms a stopper in the opening of the embryo-sac.—This apparatus is very widely distributed, especially amongst monocotyledonous plants, and projects often very far out of the embryo-sac. The longitudinal striation, often observable, arises from fine pores filled with protoplasmic contents.—We turn to our preparation lying in water or in sugar solution, and determine that the distribution of contents in the synergidæ and

oosphere is exactly the same as in *Monotropa* and *Orchis* (*B*, *C*). In the synergidæ the nuclei lie in the upper, the vacuole in the lower part; in the oosphere this is reversed.

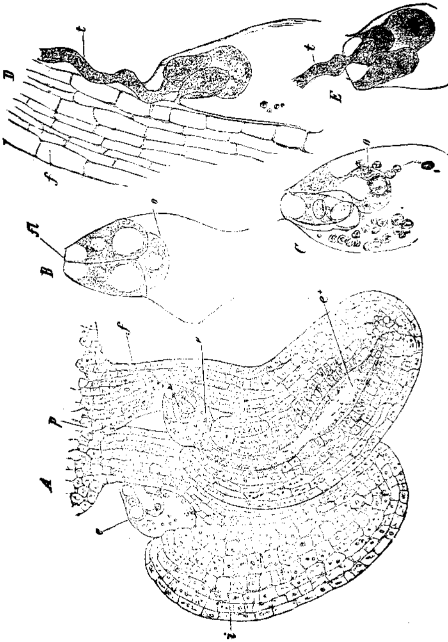


FIG. 148.—*Torenia asiatica*. *A*, two ovules on the placenta (*p*); *e*, the free apex of the embryo-sac; *e**, its broadened part in the interior of the ovule; *f*, the funiculus; *i*, the integument (× 240). *B* and *C*, free apex of the embryo-sac before fertilisation; *ſſ*, caps of the synergidæ (filiform apparatus); *o*, oosphere. *D* and *E*, during fertilisation, *D*, with a part of the funiculus, (*f*); *t*, pollen-tube (*B* to *E* × 600).

If we wish to study the process of fertilisation in *Torenia* we must pollinate the flowers for the purpose. From pollination to fertilisation about thirty-six hours elapse, so that we must renew our observations after from a day and a half to two days. As before, we free the ovules from the placenta, but as carefully as possible, under the simple microscope, in order to remove the

largest possible proportion of the pollen-tubes. These are followed here, with the greatest possible ease, up to the apex of the embryo sac, between the caps of the synergidæ, and right up to the oosphere (*D*, *E*). We see that the pollen-tubes, "conducted" by the placentæ, are still further guided by the funicles till they reach the apex of the embryo-sac. A direct influence makes itself felt at the same time from this latter, which determines the direction of growth of the apex of the pollen-tube. It can be assumed that the synergidæ secrete some definite substance which acts chemotactically as a stimulus upon the pollen-tube. The caps of the synergidæ, on account of their soft consistence, oppose little resistance to the escape of the secretion. Where the caps of the synergidæ are specially strongly developed, they appear, moreover, traversed by very fine canals, which conduct the secreted substance outwards. The synergidæ in *Torenia*, as elsewhere, become disorganised after the entrance of the pollen-tube, and take on the strongly-refractive appearance already known to us.—For the study of the further developmental stages of the embryo, etc., this object is not favourable.

One of the easiest plants in which to see the passage of the pollen-tube into the micropyle is a speedwell, *Veronica serpyllifolia*, very common in shaded grass and in hedgerows. Take a flower from which the corolla has just fallen, place the ovary on a slide in a drop of 3 per cent. sugar solution, and dissect out the ovules under the microscope or under a simple microscope. If the ovules are carefully separated, and a cover-glass put on, some of them will probably be found to show the pollen-tube in the micropyle.

NOTE ON "DOUBLE FERTILISATION" (p. 403).

While tracing the stages of fertilisation in *Monotropa*, or its substitute, it may be possible, though with difficulty, to make out a small nucleus, resembling the spermo-nucleus in the egg (*D*), attached to the side of the secondary nucleus of the embryo-sac. This is the nucleus of the second generative cell of the pollen-tube. It coalesces with the secondary nucleus of the embryo-sac, which is thereby capacitated for active segmentation. In appearance this process resembles that of fertilisation of the egg, and its discovery (by Nawaschin) gave rise to the conception of a double process of fertilisation in Angiosperms, affecting the embryo and the endosperm respectively. Certain phenomena in hybridism, already well known, are explained by it.

CHAPTER XXX.

STRUCTURE OF THE SEED OF ANGIOSPERMS.

MATERIALS USED.

Flowers and seed-pods of various ages of the Shepherd's Purse (*Capsella Bursa-pastoris*). March to November. Fresh, also in alcohol.
The same of the Water-Plantain (*Alisma Plantago*). June to August.
Ripe seeds of the Castor Oil plant (*Ricinus communis*).
Ripe grains of Wheat. Fresh; slightly softened, or, preferably, just ripe.
Germinated Wheat; fresh.

REAGENTS USED.

Potash—Carbolic acid.

Dicotyledonous Seed of Capsella.—We will now endeavour to become acquainted with the structure of a ripe seed, and to give especial attention to the embryo which it contains. As a comparatively favourable plant, we select one of the Cruciferæ, *Capsella Bursa-pastoris*, the shepherd's purse, a plant which has been very largely used for embryological studies. Its seed is comparatively small, but offers special advantages for developmental investigation. For this reason, therefore, we will endeavour to overcome the difficulty which cutting it presents. It is advisable first to prepare a *median longitudinal section* through the seed, as we wish to know what the object looks like —the development of which we are about to study. If we have fresh seeds at our disposal this section offers no insuperable difficulty to preparation between the fingers. It is still easier if we place the seed between two flat pieces of cork, and draw the razor between them. Or the seed can be fastened with gum in the desired position between two pieces of soft lime or poplar wood; and after it is dry the sections made through wood and seed at the same time. Or the seed can be embedded in a drop of gum, to which a little glycerine has been added, at the end of a

piece of elder-pith, and, after drying, gum and seed can be cut together.

The sections, in whichever way prepared, should be examined in glycerine, as water makes the embryo swell, and come out of the spermoderm. The embryo (Fig. 149, *A*), fills the entire seed; it is bent at its mid-length, so that the **cotyledons** (*c*) lie alongside the **hypocotyl** (*h*), *i.e.*, are **incumbent**. (Compare the figure.) This disposition is characteristic of several tribes of the Cruciferæ, by some systematists collected into the section Notorhizeæ, and may be represented by the sign **| | O**. Another characteristic method of folding of the embryo in Cruciferæ, is where the hypocotyl is folded over and applied to the edges of the cotyledons. This is called **accumbent**, and may be expressed by the sign **= O**. They may likewise be represented by **C C H** and $^{C}_{C}$ **H** respectively. If the section is delicate and has cut the seed perfectly in the centre (as in the adjoining Fig. *A*), we see at the base of and between the cotyledons the small growing apex (*punctum vegetationis*) of the stem, the **plumule**, and can also see, at the lower end of the hypocotyl, the axis closed by the **radicle**, covered by a **root-cap** only a few cells thick. The **endosperm** in this seed is represented only by a single layer of aleurone-containing cells. The grains which they contain take a yellow colour with iodine, and thus betray their nature. The aleurone layer immediately surrounds the embryo, and is itself surrounded by the **spermoderm**, the so-called **testa**. If we use a somewhat higher magnifying power we can determine that this spermoderm (Fig. 149, *B*) consists of three layers of cells; an innermost membranous layer (*) owing its origin to the crushed inner integument; a second layer (*c*) the cell-walls of which are coloured yellow, and towards the inner side are very strongly thickened; and an outerost layer (*e*) of cells, which appears in concentrated glycerine as olourless, apparently homogeneous membrane, while its cells

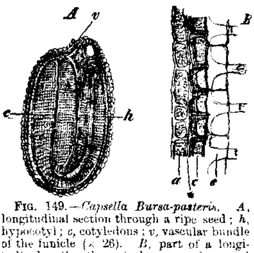


FIG. 149.—*Capsella Bursa-pastoris.* *A*, longitudinal section through a ripe seed; *h*, hypocotyl; *c*, cotyledons; *v*, vascular bundle of the funicle (× 26). *B*, part of a longitudinal section through the spermoderm and the aleurone layer, after the action of water; *e*, the swollen epidermis; *c*, the yellow strongly-thickened layer; * the crushed layer of cells; *a*, the aleurone layer (× 240).

are strongly flattened, and thickened almost to the obliteration of the cavity.—If we examine the spermoderm from the outside, we easily recognise the contour of the polygonal cells of the external tabular layer. These cells have their more internal portions partly separated by intercellular spaces full of air. In the middle of each cell is to be distinguished a weakly-defined, more strongly-refractive portion. The walls of the next inner layer of cells are brown, strongly thickened—the cells themselves only a little smaller than in the outer layer. Considerably smaller, on the other hand, and weakly thickened, are the cells of the aleurone-containing endosperm layer.

If we now allow water to run into the section from under the edge of the cover-glass, we see the cells of the outer layer rapidly swell; each bulges strongly outwards; at its centre a highly-refractive column is noticeable. The cell-cavity is not distinguishable; the entire cell appears filled by the thickening layers of the wall, and in all cases the outer thickening layers are feebly, the inter highly, refractive. These internal thickening layers form the striking central column, which now projects very strongly on the surface of the seed, while simultaneously the intercellular spaces between the cells disappear. The swelling walls usually show clear lamination.—With further addition of water, the cuticle of the cells is ruptured, and the outer thickening layers come out, diffusing in the surrounding water as invisible mucilage. The refractive column remains behind, marking the centre of each cell (Fig. 149, *B*, at *e*). It has increased not inconsiderably in size; at its apex can be seen the relics of the dissolved thickening layers. In the same way the lateral middle lamellæ of the cells remain, and, as they do not swell, show now a much less height than the columns. All this can be seen in our Fig. 149, *B*, which represents the testa after the action of water.—These phenomena of swelling can be observed more quickly if the section is first examined in alcohol, and then water run in. This mucilagination of the thickening layers of the outer cells of seeds and mericarps [1] is a comparatively common phenomenon, which facilitates the fixing of the seed in the soil; and, on the other hand, results in the firm retention of water on the surface of the seed.

[1] *Mericarps*, the segments of fruit which, like the Pelargonium (Geranium), Cranes' Bills, and Parsley Worts, do not dehisce to let out their seeds, but split up bodily into seed-containing segments. Hence these fruits are called *schisocarps*—spitting frulits.—[Ed.]

As section-cutting of ripe seeds is difficult, we can, so far as informing ourselves as to the position and structure of the embryo is concerned, prepare sections from seeds which are not quite ripe, and far softer, and only study the testa upon fully-ripe seeds.

Development of Embryo.—Now let us go back to younger stages, and at first lay the entire ovule in potash. These ovules can be best obtained by opening the ovary in its entire length, and removing the ovules from each half with the scalpel. The ovules, almost to the fully-ripe stage, can be made so far transparent that we can readily observe the position of the embryo. In potash the embryo becomes a beautiful green; this arises from the swelling of the starch-grains, so that the chlorophyll grains become visible. — Proceeding to progressively younger ovules, we see that the embryo (and at first especially its cotyledons) becomes continually shorter. It withdraws farther and farther from the lower, outward-bent, half of the embryo-sac. Ovules from ovaries which, without stalk, measure about $\frac{1}{8}$ inch in height, show the embryo as a small body of cordate form. The two divaricating anterior protuberances are the rudiments of the cotyledons.—As we trace back the various developmental stages of the embryo, we can at the same time determine that **endosperm** is formed only at the two ends of the embryo-sac, and appears, especially at the chalazal end, as a small green-coloured mass of tissue. This endosperm is displaced, and absorbed, by the growing embryo, with the exception of the outermost layer, which persists as an aleurone layer. We can also see that the spermoderm is derived from the two cell layers of the outer integument, while the cells of the inner integument are stretched and ultimately crushed.—If immature seeds of various ages are laid upon an object-slide in a drop of potash, and covered with a cover-glass, and then subjected to carefully-controlled pressure with the handle of the needle-holder, the ovules will burst, and the often uninjured embryos will come out. This process is effective with embryos from a very young state up to those that are well-nigh fully formed.

In order to inform ourselves as to the structure of the egg-apparatus in the ovule at the receptive period, we must have recourse to alcohol material, studying ovules of various ages, which we make transparent to the desired degree by careful addition of potash. We can thus state the presence of two

synergidæ, and an oosphere, in the egg-apparatus, while the antipodal cells are very difficult to see. We can see that the fertilised oosphere (oospore) grows into a thread-like pro-embryo, about six cells long, of which the uppermost cell, *i.e.*, that most removed from the micropyle, rounds off into a spherical **embryo-cell**, and the other cells collectively form the **suspensor**, while the lowermost cell of this embryo-bearer or suspensor, the attaching-cell, swells at the same time bladder-wise, absorbs the entire nucellar tissue up to the integument, and forms the bladder which we find at this place even in the ripe stage (compare Fig. 149, *A*, at the tip of the radicle). This swollen cell may aid in the absorption of nutriment for the embryo. The spherical embryo-cell is already at this stage separated from the suspensor by a partition wall, and soon after is divided by a longitudinal wall, at right angles to which follows a second longitudinal wall, and then at its mid-height a cross-wall. The still spherical embryo thus appears divided into octant cells, in which are subsequently formed periclinal and anticlinal walls. The embryo increases in size and in number of constituent cells, flattens somewhat, and then from its anterior end the cotyledons grow out. These at first are in contact at their base, and then subsequently the growing point or plumule of the stem bulges out between them.—All of these stages can likewise be followed in fresh material, and *in situ*, by laying the ovules in glycerine, covering with a cover-glass, and then carefully heating over a spirit or gas-flame. The ovule is thus made transparent, and the embryo clearly visible.—By careful use of the potash method of treatment, and cautious pressure upon the cover-glass with the needles, even the youngest embryos can be squeezed out from the ruptured ovules, though in this method the relations of the embryo with the ovule are, of course, lost sight of.

Monocotyledonous Seed of Alisma.—For the study of the **monocotyledonous embryo** we select the common water plantain, *Alisma Plantago*, which, like *Capsella*, is highly suited to this kind of investigation. We will here restrict our study to the fully-developed state. The flower of *Alisma Plantago* contains numerous monocarpellary pistils; it is **apocarpous**, resembling in this respect the Ranunculaceæ. The ripe carpels of the flower are closely pressed together in a single whorl into a collective or aggregate fruit—a dry **etærio** of triangular outline. Each individual ripe carpel (**achene**) is strongly flattened laterally, some-

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what thicker above, obovate in profile, with a median dorsal groove. At about mid-height of each achene, on its internal (ventral) edge, is a short thread-like outgrowth, representing the withered style, which, therefore, is ventral.—For further study we select an almost ripe etærio, place a single achene between the two halves of a split cork, and draw the razor between these two halves. We shall thus, without trouble, obtain tolerably median *longitudinal sections*; while cutting between the fingers presents difficulties, as the spermoderm is very hard. At the same time we will prepare some cross-sections between two pieces of cork. The longitudinal sections we examine in water, to which we add a little potash. For cross-sections pure water suffices. For the study of the spermoderm by means of longitudinal sections the air must be first driven out; for this we can either lay the sections for a short time in alcohol, or else place them under the air-pump. We also lay some longitudinal sections in carbolic acid, and in this way obtain figures which supplement the others in the most satisfactory way.

The *longitudinal section*, if correctly cut, presents the appearance of the adjoining Fig. 150. We have first a comparatively thick wall to the fruit, the **pericarp**, which in its surface is covered by the epidermis (*ep*). This is, as our median longitudinal section teaches us, a pretty sharply delimited part of the pericarp, and is therefore distinguished as **epicarp**. To the epidermis follows a parenchymatous tissue of approximately isodiametric cells, strongly thickened, filled with air, and without intercellular spaces; this forms the **mesocarp** (*m*). To it follow several layers of elongated sclerenchymatous elements—the **endocarp** (*en*). A quite median longitudinal section cuts, at the back of the pericarp, a mucilage passage adjoining the epidermis, which is, however, only to be seen well in an unripe pericarp; in a ripe one, on the other hand, it appears almost empty, and can scarcely be distinguished from the neighbouring tissue. Longitudinal sections not truly median may, on the other hand, expose a vascular bundle (*v*), which, adjoining the sclerenchymatous endocarp, passes up at the back of the fruit, in order to end in the lower half of the ventral edge (at *v**). Under the place of insertion of the withered style (*st*) the ventral edge of the epicarp projects, and is here formed of elongated cells. Adjoining these towards the interior we see, in favourable cases, an air-passage (*t*), which, continuing the pollen-passage through the style, can be followed to the base of the cavity of

the ovary. This is the passage by which the pollen-tubes arrive at the micropyle. As the ovule turns its micropyle to the dorsal edge of the ovary, the pollen-tubes, after entering the cavity of the ovary, must grow round the funicle.

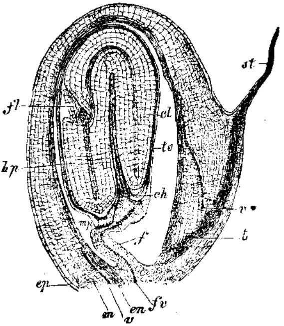

FIG. 150.—*Alisma Plantago.* Median longitudinal section through a ripe achene; *ep*, epicarp (epidermis); *m*, mesocarp; *en*, endocarp of the wall of the fruit. *v*, a vascular bundle in this; *v**, the end of the vascular bundle; *st*, the withered style; *t*, the pollen-passage; *f*, funiculus of the seed, with its vascular bundle *fv*; *mp*, micropyle; *ch*, chalazal end; *ts*, the spermoderm; *hp*, hypocotyl of the embryo; *fl*, first leaf; *cl*, cotyledon (× 28).

The seed almost completely fills up the cavity of the ovary, and is fixed in a median position at the base of the cavity of the ovary by a tolerably long, bent funicle (*f*). A vascular hundle enters the funicle. The seed is campylotropous, and completely filled by the embryo. As spermoderm (*ts*) only a thin skin is present, consisting of two clearly distinguishable layers of cells. Between the two we see here and there also a third crushed layer, which comes out more clearly after swelling in potash. The inner cell layer of cells of the spermoderm is strongly thickened on the inner side. The micropyle (*mp*) is very prominent on the seed. The radicle of the embryo lies directly against the inner side of this. The radicle is somewhat swollen, and projects nipple-like in the middle. If the section has cut the embryo quite medianly, we see that this nipple-like projection is formed of two layers of root-cap, which at the edges pass over into the epidermis. At mid-height of the seed is to be seen upon the embryo an outward-turned narrow notch, in which lies the growing apex of the stem (plumule). This growing apex is surrounded by the cotyledonary sheath. Upon the apex arises a rudimentary leaf (see figure, towards the left hand) in the middle line of the outer side, which completely fills up the notch. The part found between this growing apex and the end of the root is the hypocotyl. It is

covered by the epidermis, shows usually three layers of **cortical cells**, *regularly arranged into a cylindrical sheath*, and a *median* string of elongated cells which runs from the root-apex towards the growing point of the stem. The cortical layers have at the apex only one layer of common **initial cells**. Over these runs the **dermatogen**, from which the two layers of root-cap appear segmented. The central string, the **plerome**, is terminated by its own initials. The **hypocotyl** is prolonged into the single **cotyledon**. This is bent, corresponding to the form of the seed cavity, tapers gently to its apex, and ends at the chalazal end of the seed. The cotyledon also consists of a cylinder of regularly arranged layers of cells, and is traversed by a central string of elongated cells. This string curves under the **plumule**, and joins with that of the hypocotyl (see figure). The cell-rows of the cortex also pass over from the hypocotyl into the cotyledon with a gentle curve. The cotyledon in its lower part, like the hypocotyl, has three, higher up, as it tapers, two, and finally only one layer of cortical cells. The central string ends at some little distance from the apex of the cotyledon. In the ripe seed there is not a trace of endosperm. This seed likewise is exalbuminous. The embryo itself has all its cells densely filled with starch.

The *cross-section* of the seed shows nothing new. It presents simultaneously two cross-sections of the embryo, separated by a narrow strip of tissue which passes over into the inner cell-layer of the spermoderm. The structure of the spermoderm is more clear than in longitudinal sections. The cross-sections of the embryo show the concentric arrangement of the cortical layers very beautifully. The division of the pericarp into epi-, meso- and endocarp is even easier to recognise in cross than in longitudinal sections; and the furrow in the middle line of the back is now very noticeable.

The two angiospermous plants investigated by us offer us truly typical but extreme examples of embryo formation in dicotyledons and monocotyledons respectively; types which are far from exhausting the whole diversity of cases which have been investigated. Thus amongst Dicotyledons are examples of embryos which have only one cotyledon (*Carum Bulbocastanum*, the Pignut, *Ranunculus Ficaria*, the Pilewort); and amongst Monocotyledons those in which the cotyledon arises laterally from the growing apex of the stem (Dioscoreaceæ, Commelynaceæ). Lastly there are seeds in which the embryo persists as a few cells only.

The two cases studied above are both examples of **exalbuminous seeds**, *i.e.*, of seeds in which the endosperm formed in the embryo-sac is completely, or almost completely absorbed into the embryo during its early growth, and the reserved food stored up mostly in the cells of the cotyledons. Examples of the other primary, but less common, type of seed, known to systematists as the **albuminous seed**, *i.e.*, in which the endosperm formed in the embryo-sac is not absorbed by the embryo until the period known as germination, have been already in part studied by us in Chap. II., especially in the case of *Triticum* as a Monocotyledon, and *Ricinus communis*, the Castor Oil, as a Dicotyledon. It is advisable for the student to amplify his knowledge of the structure of seeds by a careful study of the ripe seed of this latter plant, first removing the outer spermoderm, or **testa**, as it is too hard for satisfactory section-cutting. Within this testa is a membranous inner spermoderm—the **tegmen.** The embryo is straight; the two large, delicate, veined cotyledons lie on either side of a narrow central cavity, which nearly separates the seed into two halves; the radicle projects towards the curious, wart-like **micropylar aril** which will have been noticed at one end of the testa. The embryo is embedded in oily endosperm. The information given in the above chapter will enable the student to fill in the details of the structure of the seed.

The Grain of Wheat.—On account of its special interest, we will, however, make a full study of the "**grain**" **of wheat**, the fruit of *Triticum vulgare.* The ovary of the flower of the wheat plant is superior, or free, and contains a single ovule. This latter, in its development, completely fills the cavity of the ovary, the pericarp adhering to the spermoderm, as is shown in Fig. 12, p. 30, and both being strained and flattened, so that the constituent cells collapse. We may investigate either softened, or, what is even better, just ripe grains. If we take softened grains, we must take care that the grain is only just soft enough to make it suitable to cut. The ripe grain of wheat shows in the middle line of its inner side a deep furrow, corresponding with the ventral suture of the ovary. At the base of the opposite side is the embryo, recognisable as a slightly elliptic protuberance ending conically below. The flattened top of the grain is crowned with hairs, between which is the filiform residue of the style. The "grain" of wheat is not a naked seed, but a one-seeded, dry, indehiscent fruit—a **caryopsis.**

Take first of all *cross-sections* of the grain at about mid-height, and examine them in water or in glycerine, and subsequently with the addition of a little potash. Leaving the somewhat complex structure of the furrow out of the question for the present, we find that the pericarp is composed externally of one or several layers of thickened pitted cells, the walls of which are highly refractive and yellowish, and colour a deep yellow in potash. The outer-

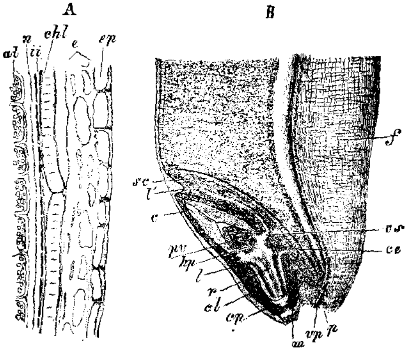

FIG. 151.—Grain of *Triticum vulgare*, the wheat. *A*, cross-section through the pericarp and testa. Of these, *ep* is the epicarp (epidermis); *e*, outer layers of the mesocarp; and *chl*, chlorophyll layer; these collectively constituting the pericarp; *ii*, remnants of the ovular integument; *n*, the outermost thickened layer of the nucellus; *these together constituting the testa*; *al*, the aleurone layer of the endosperm (× 240). *B*, median longitudinal section through the lower part of a ripe fruit. At the bottom of this, to the left, is the embryo with the scutellum, *sc*; *l'*, the tooth-like development of the scutellum; *vs*, its vascular bundle; *ce*, its "cylinder epithelium"; *c*, the sheath of the cotyledon; *pv*, the growing apex of the stem; *hp*, the hypocotyl; *l*, the epiblast upon it; *r*, the radicle; *cp*, the root-cap of the radicle; *cl*, the root-sheath (coleorhiza); *m*, place of exit of the radicle, corresponding with the micropyle of the ovule; *p*, the funicle; *vp*, vascular bundle in the funicle; *f*, side wall of the furrow (× 14).

most layer of these cells is the epidermis (Fig. 151, *A*, *ep*), the succeeding layers (*e*) belong to the inner tissue of the pericarp, the innermost layers of which are for the most part obliterated. To this outer tissue succeeds a layer of tangentially-elongated, straight, or more or less curved, cells (*chl*), marked by numerous narrow, cross-set pits. Now and again on the inner side of this pitted layer we see sac-like cells, which indicate the inner epider-

mis of the pericarp. This is all that remains of the pericarp. The tissue next following appertains to the seed. It is separated from the pericarp by more or less numerous air spaces. The **spermoderm** shows first a thin, apparently homogeneous, colourless skin, arising from a layer of cells with obliterated cavities; to this follows a similar narrow layer, the scarcely recognisable cavities of which have brown contents. These two layers are represented in *ii*, in Fig. 151, *A*; together they constitute the spermoderm; the outlines of their cells are recognisable upon tangential sections. All the elements of pericarp and spermoderm are, so far as they possess cavities, filled with air. To the spermoderm succeeds a relatively thick, strongly refractive, white skin (*n*), which owes its origin to the outermost layer of the nucellus. The original cavities of the cells in this are indicated by narrow, granular, tangential striæ. To this layer follows the layer of radially-elongated aleurone-containing **endosperm cells** (*al*), which we have already studied in Chap. II.; and next to these come the cells of the inner endosperm, filled with starch.—The wall of the furrow shows the mesocarp much increased in quantity, and the layers progressively of larger cells; while in the middle line the elements are again small, thin-walled, without interstices, and have a median, feebly-developed vascular bundle. The layer *chl* is thicker, and contains chlorophyll and large air-cavities; the outer nucellar layer thickens into a cushion of tissue, behind which the aleurone layer is often wanting. This increased development of the outer tissues is accompanied by a deeper grooving of the endosperm than of the spermoderm aud pericarp, and a sharp limit between the tissue of the seed and of the fruit is not recognisable in the furrow.

Let us now endeavour to obtain a tolerably *median longitudinal section* through the ripe grain, for which purpose we must not, however, use air-dry, but softened, or even better, grains which have only just ripened. The embryo will show specially well in sections examined in carbolic acid, or treated with potash and examined in glycerine. We will first examine the section with a low power, and only use a high power for the detailed study of individual parts. We begin with the **embryo.** This is situated obliquely, at the base of the body of the endosperm (Fig. 151, *B*), and is in contact with it by means of the shield-like **scutellum** (*sc*). The scutellum appears in longitudinal section as a flat structure, which at both upper and under termi-

nation ends free in a blunt projection. Bounding the scutellum in the upper half of the embryo is the tube-like **cotyledonary sheath** (*c*). This sheath surrounds many rudiments of leaves, which decrease in size progressively inwards. The largest of these leaf-rudiments stands outwards in the middle line. Between the youngest (innermost) of the leaf-rudiments lies the growing apex, from this point of view appearing relatively narrow and tapering (*pv*). Together with the leaf-rudiments this forms the rudimentary bud, the **plumule.** The plumule and the cotyledon are borne upon the stem, or **hypocotyl** (*hp*). The hypocotyl is prolonged below into the **radicle** (*r*), which is directed somewhat obliquely forwards. Even with low magnification this shows the central **plerome**, closed cone-wise towards the apex, and surrounded by the **periblem** and **dermatogen.** At the apex the *periblem and dermatogen come together into a single layer of* cells (compare with this Fig. 87, p. 221). Inside the plerome-cylinder the rudiments of the first vessels can be very easily seen, and traced right up to its apex. The **root-cap** (*cp*) lies over the apex of the root as a bright cover. The whole of this rudimentary root lies in a closed sheath, the **coleorhiza** (*cl*), and is sharply limited towards it by a clear line, which indicates the thickened walls of its dermatogen cells. This clear line disappears over the root-apex, between the body of the root and the root-cap. At its base this root-sheath passes over into the tissue of the hypocotyl. At its part which ensheaths the root-apex the coleorhiza is swollen into a clearly distinguishable wart-like projection (compare the figure); at its upper edge, where the root-sheath passes over into the hypocotyl, is developed a free outward projection, the **epiblast** (*l*). A string of elongated cells (*vs*) can be traced from the hypocotyl into the scutellum; the epidermal cells (*ce*) on the outer surface of the scutellum are distinguished by their considerable radial elongation. The scutellum is a sheath-like outgrowth from the base of the cotyledon, and must therefore be looked upon as a part of it. It remains in the seed during germination, and serves as a suctorial organ. The absorption of the food-materials takes place by means of its cylindrical epidermal cells (epithelium), and continues until the whole of the reserve food-materials of the endosperm are exhausted.

The embryo lies in immediate contact with the spermoderm, while the **pericarp** is here somewhat thicker, but more loosely constructed. Under the wart-like apex of the coleorhiza, *i.e.*, at

the place from which, in germination, the radicle will protrude, it shows still further reduction and a depression (*m*) is visible. The fruit has a very short stalk (*p*), and we can see the vascular bundle (*vp*), which enters here and passes into the funicle of the seed, which is fused, however, with the pericarp, and the vascular bundle is hardly recognisable. Farther inwards, however, can be seen a much more noticeable string of grey-brown elongated, slightly-pitted cells, which we have already noticed in the cross-section. The vascular bundle itself is embedded in delicate walled, colourless, slightly-elongated cells. Farther inwards are the layers of nucellar cells, with fairly thick, white walls, and then comes the aleurone layer of the endosperm. This is readily separable from the nucellar cells, so that an air-space is often present at this part. Towards the embryo the endosperm is not bounded by an aleurone layer, but by a fairly thick layer of swollen cell-walls, which represent endosperm cells which have been crushed back by the enlarging embryo.

Germination of Wheat.—The ripe grain of wheat germinates very easily, and we will therefore make use of it in order to study the early stages of germination. It will suffice to lay ripe grains in moist sawdust; it is even enough to stand ripe ears for a few days with their lower ends in a glass of water. The pericarp of the grain is first of all broken through at its weakest part (*m*), that which corresponds with the micropyle of the seed; the coleorhiza is protruded, and out of its tip the rapidly-lengthening radicle soon projects, the coleorhiza surrounding its base like a sheath. Above this point the lowermost pair of lateral roots subsequently arise, each likewise surrounded at its base by a root-sheath, or coleorhiza. The whole grain swells considerably, and ruptures the pericarpic covering more or less completely. If this is lifted off, we can easily see, with the lens, the epiblast between the bases of the two lateral roots. The cotyledonary sheath elongates, and assumes a greenish colour. After it has attained about fifty-fold its original length, it is broken through at its apex by the first bright green foliage leaf. Considerably later than the first, or lower, pair of lateral roots is produced a second, upper pair. The original distance apart of the structures in the neighbourhood of the lateral roots remain unchanged, and shows that the hypocotyl undergoes no considerable elongation. The lateral roots soon equal in development the principal roots, a tap-root is therefore not produced.—From a seedling which has already

produced all its rudimentary roots, we now cut off all the elongated parts, and prepare a median longitudinal section through the fruit. We can now easily see that the growing point remains pretty much in its old position, having only developed a number of new leaf rudiments. The scutellum has in general not increased in size, excepting as to its cylindrical epithelium, the cells of which have elongated still more, and more or less completely separated from one another laterally, so as to resemble hairs; they have rich protoplasmic contents.—It is of great interest for us now to take a little of the endosperm tissue, diffuse it in a drop of water, and examine it with a high power. Amongst the more or less numerous starch-grains which are still unchanged will appear others which have become corroded by the action of diastatic ferments which have been produced in the course of germination. Such grains appear peculiarly changed. In places still white, of their original density, and without clear lamination, they are in other places transparent, sharply laminated, and with the concentric layers traversed by more or less broad radial striæ. Many grains appear as if reduced thereby to verniform particles, and finally such grains are completely dissolved.

CHAPTER XXXI.

THE FRUIT OF ANGIOSPERMS — MUCILAGES — DEVELOPMENT OF THE FLOWER.

MATERIALS USED.

Ripe Plum or Cherry. Ripe Apple. Ripe Orange. All fresh.
Young inflorescences of *Brassica Napus*, the Rape; fresh.

REAGENT USED.

Potash.

ONE of the simplest possible fruits, the achene of *Alisma Plantago*, we have already become acquainted with; as also the almost equally simple caryopsis of the wheat. We will now take one or two cases in which special modifications in development lead to ultimate complexity of structure.

The Plum or Cherry.—A ripe plum, *Prunus domestica*, using any firm-fleshed kind, shows upon its surface a delicate covering of wax, the so-called "bloom," which, in a *surface section* of the epidermis, appears as a finely-granular covering. The same section shows the epidermis composed of cells, which are arranged into groups, and thus betray their origin from common mother-cells. They contain rose-red cell-sap.—A delicate *cross-section* shows us under the epidermis some layers of cells rapidly increasing in size, and, farther in, cells which are all uniformly large. These are rounded off from one another, but form only small intercellular spaces. They contain very small, scattered, yellowish-green chlorophyll grains, a thin peripheral layer of protoplasm, a nucleus, and colourless cell-sap. This parenchymatous tissue is traversed by numerous branching vascular bundles, and towards the stone the cells become smaller, radially elongated. The stone itself, which, in order not to chip the razor, must be cut with the greatest possible care, attempting very small sections only, and on a surface previously prepared with a strong pocket-knife, consists

of very strongly-thickened and lignified elements, the walls of which are traversed by beautifully-branched canals. The history of its development shows that the stone belongs to the wall of the ovary, the **pericarp**; that the epidermis of the plum, the **epicarp**, arises from the epidermis of the ovary; the **flesh** of the fruit, the **mesocarp**, arises from the outer tissue of the ovary, underlying the epidermis; and the stone, or **endocarp**, from its inner tissue. The entire tissue of the plum, including the shell of the stone, owes its origin, therefore, to the wall of the ovary.—Enclosed in the stone is the **seed**, consisting of the **embryo**, a delicate **spermoderm**, and a mere remnant of **endosperm** remaining between the embryo and the spermoderm, so that morphologically the seed is **exalbuminous.** If we cut it across we can easily distinguish the two **cotyledons**, lying flat together. A *median longitudinal section* of the seed shows us, at its base, connecting the two cotyledons, the **hypocotyl** of the embryo, with its **radicle** projected into the pointed micropylar end of the seed, and, between the base of the two cotyledons, the bud or **plumule.** The embryo, during its enlargement, has absorbed almost the entire tissue of the ovule, up to the very thin spermoderm, from which still arises, quite close to the micropyle, the withered funicle.

A delicate *cross-section* through the seed shows us the **spermoderm** composed of layers of collapsed cells, and covered on its outer side with rounded cells, thickened either only or chiefly upon the outward bulging sides, and standing either singly or in groups. *Surface sections* of the spermoderm show us that the thickened projecting elements are single cells or groups of cells of the spermoderm. These have been thickened, and are resistant, while their neighbours remain unthickened, and have collapsed. The pits, abundant upon the side walls, give to these cells an elegant appearance. Where two thickened cells are in contact, the pits coincide. To the epidermis of the spermoderm follows a layer consisting of flattened, irregularly-thickened cells. On the two broad sides of the kernel, a multilamellar layer of endosperm is present, separating the kernel from the spermoderm, but on the narrow sides this is reduced to a single cell layer. Two ovules are present in the ovary, of which only one is developed further. —Such a free monocarpellary fruit as the plum, with succulent mesocarp, and a stony endocarp, is known as a **drupe.**

This description of the plum will, apart from unessential differences, serve likewise for the cherry, which therefore can be

studied in the place of the former. This enlarges the opportunity of examination in the fresh state, since cherries or plums can be obtained, more or less ripe, from April, or even earlier, till October, inclusive, and can be readily used for studies in the development of the various tissues, especially of the endocarp in the phenomenon of "stoning," which takes place at a definite period of their growth, during which, for some little while, the young fruit does not increase in size.

Structure of the Apple.—The apple, like the plum and cherry, is a **fleshy indehiscent fruit**; while, however, the plum or cherry owes its origin to a free or superior ovary, formed from a single carpellary leaf, the apple arises from an inferior, five-celled ovary, composed of five carpellary leaves. However, having regard to the relations which the nearly allied roses offer, we can assume that the five-celled ovary here is immersed in a hollowed flower-stalk, a so-called **hypanthium**, or **receptacular tube**, and is adnate to this; a view capable of support, however, only on philogenetic grounds. The apple is crowned by five more or less completely-shrivelled sepals, and also by the withered relics of the rest of the floral parts. *Surface sections* show the epidermis of the apple to be composed of comparatively small polygonal cells, upon which grouping, as a result of development, can still be recognised. The walls of the cells are pretty strongly thickened, their cell-sap either colourless or red. The surface of the epidermis is covered with a finely granular covering of wax. The small prominences, which are readily visible on the surface of the apple with the lens, are occupied, each in its centre, by a stoma. The tissue under such a stoma is often dead, or else the epidermis is here ruptured, and the wound closed with cork.

Thin *cross-sections* show us that the epidermis is strongly thickened on its outer side. Below it lie several layers of tangentially-elongated cells, with tolerably thick walls, which, passing inwards, gradually become larger and thinner walled, and at the same time chlorophyll-containing; otherwise no sharp limit between epicarp and mesocarp is present. The chlorophyll grains are densely filled with starch; their colour dies away towards the interior of the apple, they at the same time become less numerous; at length, at a certain depth, the large bladdery cells of the mesocarp contain, besides the delicate peripheral layer of protoplasm and nucleus, for the most part only colourless cell-sap; the intercellular spaces are here filled with air. Vascular

bundles are scattered in the entire tissue. The five cells, forming the "core," are covered each by a smooth, hard, cartilaginous membrane, the endocarp. This corresponds with the shell of the plum-stone. It consists of several layers of sclerenchyma fibres, thickened almost to the obliteration of their cavity, and the thickening layers of which are pierced by fine pores. Surface sections show that these sclerenchyma fibres slope irregularly, often are bent, and in the different layers have oppositely inclined courses. The five cells often separate in the middle, forming a central cavity, into which the individual cells then usually open. *At the base of each cell are inserted two ovules, of which both, or* only one, produce seeds, or of which often neither develops further.

The **seed** is almost filled by an embryo of the same structure as in the plum or cherry. The brown **spermoderm**, on the other hand, is much thicker than in the last-named plants. In *cross-section* it shows an epidermis, the cells of which outwardly are strongly thickened, the external layers colourless, and capable of strongly swelling, the inner brown coloured and not swelling. In sections laid in water the swelling layers, increasing in volume, at length break through the cuticle, and project outwards like papillæ. *It is these which make the wet seeds slippery.* The thick tissue underlying the epidermis appears in cross-section to be formed of polygonal cells, rounded at the angles, brown, and pretty strongly thickened, and to it succeeds a layer of cells only about one-third so thick, tangentially elongated, but less strongly thickened. These *impinge on a shining white, thick membrane.* This last arises from the strongly-thickened outer walls of the outermost layer of the nucellus; the entire remainder of the spermoderm comes from the outer integument of the ovule. The inner integument of the ovule is very early displaced. The nucellar cells, the thickening layers of which we have ascribed to the spermoderm, are mostly collapsed, as also are the rest of the cells of the nucellus which are present. To this collapsed layer of tissue succeeds a thin layer of **endosperm,** which ensheathes the embryo. The endosperm-cells are densely filled with aleurone grains.—As successive *surface sections* show, the epidermis consists of cells which are comparatively but little elongated, the inner thickening layers of which are pitted. The tissue following the epidermis, which in cross-section appears isodiametric, shows now *to be longitudinally elongated*, and provided with oblique

cleft-like pits. The tangentially-elongated inner elements of the spermoderm are arranged at right angles to the preceding.

Structure of the Orange.—The cross-section through a ripe orange, *Citrus vulgaris* (*C. aurantium*), shows externally the part designated **rind**, and internally the "segments" filled with the orange-red **flesh**, the number of which segments is uncertain, and varies from six to twelve. The segments are separated by thin membranes, which combine into a central column of tissue. If we wish to apply the customary designations of the parts of a fruit to the structure of this, we can speak of the outer rind as epicarp, the orange-red flesh as mesocarp, the inner column of tissue and the partition walls as endocarp.

We pass now to a microscopical study of the individual parts. In delicate *cross-sections* through the rind we see most externally a small-celled epidermis, to which succeeds a tissue of gradually enlarging cells. The epidermis and adjoining tissue contain orange-red chromatophores, which farther inwards disappear. Intercellular spaces filled with air appear here between the cells, and gradually enlarge, since the tissue itself acquires the character of a looser spongy parenchyma. The rind is traversed by vascular bundles, which the cross-section lays bare chiefly in their longitudinal course, and which branch towards the periphery. Adjoining the epidermis are the large **glands** of **ethereal oil**, visible to the naked eye. They show throughout the structure known to us from *Ruta*, and allow the inner investment of delicate cells to be readily distinguished. The fruit, observed macroscopically from the outside, shows the oil-glands as darker spots, the tissue separating them as a brighter network.

A delicate *surface-section* of the outer side shows us first the small polygonal epidermal cells. Those lying over the oil-glands are distinguished by absence of the orange-red chromatophores; they contain in place of them colourless globules of various sizes. Scattered in the epidermis are the stomata, devoid of protoplasm, and closed inwards. The next deeper sections give instructive views of the oil-glands, and terminations between them of the vascular bundles. Still deeper sections show the spongy tissue, of elongated sac-like cells. Adjoining the loculi of the ovary the cells of the rind become still longer, fibrous, in part strongly thickened and then provided with narrow, obliquely mounting pits. The partitions between the "segments" are constructed in the same way: in the interior, of spongy parenchyma; outwardly

of fibrous, in part thickened tissue. The spongy elements, found outside the loculi as well as in the interior of the partitions, easily fall out of union. The fibrous elements, on the other hand, appear pretty firmly connected together.—The best view of these latter is obtained in surface view. We separate the segments from one another in the customary way; the spongy tissue surrounding the loculi is thus torn, the fibrous layer remains, however, as a delicate white sheath around the flesh of the fruit. If we now spread out such a sheath and examine it with a high power, we see it constructed of several layers of fibres, running parallel to the surface of the loculus, and at right angles to its long axis. Between unthickened fibres are scattered others of similar form, thickened and pitted.

The **flesh of the fruit** consists of club-shaped sacs, of which it can be readily seen, even macroscopically, that they all arise from the outer side of the loculus. They are here inserted with a narrow base, and, crowded together, fill up the loculus. The larger they are the more deeply they extend into the loculus; their course is radial, at right angles to the long axis of the loculus. Each individual club shows at its surface to be surrounded by a layer of closely united elongated fibrous cells, just as we see them in the partitions of the segments. Interpolated between these cells are single ones, more strongly thickened, and provided with obliquely ascending pits. The interior of the clubs is, however, filled with very large, polygonal, delicate-walled cells, full of sap, in the interior of which are visible spindle-shaped, very narrow, orange-red chromatophores. The **central core** of tissue, in which the partitions come together, is formed of similar spongy parenchyma to the internal part of the rind. In "quartering" an orange we free, as we see, the contents of the loculi, surrounded by the fibrous layer clothing each loculus, which is easily separated from the spongy parenchyma. This fibrous layer can now be very easily separated from the sides of each segment, with more difficulty from its outer surface, because here the sacs of the flesh are joined to the fibrous layer. In the flesh the **seeds** ("pips") lie embedded in uncertain number. They occupy the inner edge of the sections, their place of insertion being turned inwards. In isolating the sections the seeds are separated from the placenta; usually, however, portions of the inner core of tissue, together with the placentæ, cling to the inner edges of the sections. Mucilaginisation of the spermoderm

of the seed is again very evident, and gives it its slippery character.

Forms of Mucilage.—The various **mucilages** which are present in the cells and in intercellular reservoirs of plants have been classified into three groups, corresponding with the three fundamental substances which take part in the composition of vegetable membranes. In this way we can distinguish **cellulose, pectose** and **callose mucilages.** The **cellulose mucilages** coagulate in a mixture of hydrochloric acid and alcohol, and then appear insoluble, and do not even swell, in a solution of ammonium oxalate, which dissociates the tissues. In water they swell slowly. They have the general properties of cellulose, stain easily with stains which colour cellulose, especially after previous treatment with potash, but hardly react to iodine compounds. Cellulose mucilages are rare ; an example is found in Salep, the mucilage of Orchids.

The **pectose mucilages,** to which the greater number of so-called true mucilages belong, swell pretty quickly in water, and are well nigh completely dissolved. The solution is viscous, and filters very slowly. Pectose mucilages are coagulated by acetate of lead, alum and sulphate of iron, do not stain in cellulose reagents, and take a yellow colour in iodine solutions. To them belong the mucilages of Malvaceæ, Tiliaceæ, Rosaceæ, the jelly of many Algæ, *e.g.*, *Nostoc*, etc.

Callose mucilages at first scarcely swell at all ; then, however, dissolve suddenly, without the interstages which pectose mucilages show. They are found in all tissues and membranes which are distinguished by rapid solution, *e.g.*, the callus of sieve-tubes, the sporangium wall of Mucorines, the cell-wall of pollen mother-cells.

With polarised light cellulose mucilage is illuminated with the crossed prisms just as cellulose is ; the other mucilages are optically inactive.

Mucilaginous mixtures are also found, especially of cellulose and pectose mucilages. Such a mixture is found in the spermoderm of *Sinapis nigra* and *S. alba*, of *Linum*, etc.

Development of the Flower.—We will now complete our conception of the nature of the floral parts by following the early stages of their development. For this purpose the Cruciferous flower is very well suited, and, while practically any species will do, we will select the Rape, *Brassica Napus*. In any case an

[1] For further reactions of the mucilages, see note at end of chapter.

examination of the fully-formed flower must precede any attempt *to study its development.* The **inflorescence** of the rape is a **raceme**, of which the apex continues to develop for some time, so that the young flowers are crowded together as into a corymb. The flowers are stalked, ebracteate. They bear four linear, greenish **sepals**, in two pairs, of which the outer are, so far as relations with the axis of the inflorescence are concerned, in the middle plane, that is, are antero-posterior, and the inner pair are at right angles to them, and are therefore lateral. In the bud the inner have their edges very recognisably covered by the outer sepals, and their respective positions are easy to determine (Fig. 152). Four **petals** follow the four sepals, and alternate with them as if the latter also formed one whorl of four. The petals are obovate, and stalked, so that we can distinguish **limb** and **claw**. After these four diagonally placed petals, come two laterally situated short **stamens**, to which follow, in the middle plane, two pairs of longer stamens. Finally comes the narrow, laterally-compressed **pistil**, gradually tapering into the **style**, and with a feebly two-armed **stigma**.

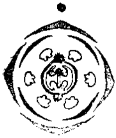

FIG. 152.—*Diagram of a Cruciferous flower.*

Cross-sections through the **ovary** show that it is bilocular; but in order to cut through the insertion of one of the (usually six) ovules it is necessary to cut through the lower third of the ovary. The partition which divides the ovarian cavity in the median plane is a false one, known as the **replum**, and the placentation is parietal, on the angles which this partition makes with the wall of the ovary. The pistil consists, therefore, of two laterally-placed carpels, which coalesce only by their edges, so that the placentæ would naturally have been in contiguous pairs upon an unilocular ovary, had the replum not been present to separate the carpels, and placentæ appertaining to them, from one another.

In order to work out the development we take the tip of a young inflorescence, and first remove from it all the larger flower-buds, up to those the height of which does not exceed $\frac{1}{25}$ inch. We then continue the process under the dissecting microscope, using no water to the object, until only the innermost flower rudiments remain. Close beneath these we cut the axis of the inflorescence across, so that they are left standing erect. We can now add a drop of water, cover with a cover-glass, and remove

under the air-pump all the air clinging between the rudiments. Brought under the microscope the rudiments are now seen in apical view, or but slightly inclined, but they are still not transparent enough to permit detailed view into their interior. We therefore run in a little potash, and can now, in the most favourable cases, see at one and the same time the most important stages of development.

The **flower rudiment** arises as a bare conical protuberance upon the axis of the inflorescence, close under its apex. No rudiments of **bracts** are visible, as the Cruciferæ generally are distinguished by the absence of bracts from the floral region. When the naked rudiment has attained a not inconsiderable height, begins the formation of the first pair of **sepals** in the median (antero-posterior) plane, the outer one originating a little earlier, and continuing somewhat more advanced; the two lateral sepals follow quickly, and quite simultaneously. All these sepals are recognisable in the form of broad protuberances, each occupying about one-fourth of the periphery of the conical flower rudiment. The growing point of the rudiment now bulges outwards a little, and, alternating with the four sepals, the four petals appear simultaneously, in the form of four protrusions, which give to the growing point a quadrangular form. The sepals now quickly close together with their apices over the rudiment, whereby the outer median (anterior) sepal overlaps the tip of the median inner (posterior). While this is going on, the two protuberances for the lateral (outer) pair of stamens appear; and then, in the median plane, the two staminal rudiments on either side. Whether these make their appearance as four isolated protuberances, or whether on the other hand they arise each pair from a previously common one, cannot be off-hand determined. This point has given rise to numerous researches, in order to show whether the stamens are isolated from the beginning, or whether we have a splitting, a so-called "dédoublement," whereby two staminal protuberances give rise to four. On theoretical grounds the latter seems the more probable. We should then have the regular alternation of two two-membered whorls of stamens, followed by the two laterally-placed carpels of the pistil, and should thus have resemblances to the nearly allied Fumariaceæ. The approximation of the large antero-posterior stamens in pairs likewise supports the dédoublement theory. It is only, however, as a matter of probability that we may conclude that the flower of

the Cruciferæ has been derived from one which bore two antero-posterior stamens.

While the petals in our object develop very slowly, the rudiments of the stamens enlarge rapidly. They are, therefore, very readily visible, while the petals are hard to see. Our knowledge of the distribution of parts in the fully-formed flower helps us to avoid confusion, and materially facilitates orientation. After the *origin of the inner staminal pairs, the apex of the floral axis* commences to bulge in the form of a two-lipped crater, somewhat laterally compressed, in the base of which the growing point must therefore be now sought. This crater increases in height but slowly, while the stamens develop very quickly, and soon form the largest structures enclosed within the sepals. The petals, on the other hand, still remain very small, and cannot be off-hand recognised within the bud when made transparent. They show more clearly, as small tongue-shaped flaps, in buds which have been carefully crushed under the cover-glass. The petals first begin to grow, and then somewhat rapidly, in buds which (without stalk) are more than one mm. high, and in which all parts of the stamens are recognisable. These relations, however, as well as the destiny of the pistillar rudiment, can no longer be followed upon entire buds, but only upon sections, or upon dissected buds.

Let us prepare *longitudinal sections* through the apex of the entire inflorescence between the fingers. In order to isolate the parts, we dissect the buds with needles under the simple microscope. The sections, as well as the separated parts, can with advantage be treated with potash. We can then determine that the deeply two-lipped rudiment of the pistil, after it has attained a certain height, begins to close above; that, simultaneously with the lower parts of the ovary, a partition wall arises from the *punctum vegetationis*, and thus divides the ovarian cavity into halves; that finally out of the angles on each side of this partition wall three **ovular rudiments** arise. The **placentæ** are therefore at the angles of this partition wall. The ovular rudiments are at first conical and not bent; they produce under their apex an annular wall-like prominence—the **inner ovular integument**; they then begin to bend, while at the same time on their posterior surface a second protuberance, the **outer ovular integument**, arises close under the first. While this increases in strength, the ovule bends more and more. The integuments, growing by their

upper edge, reach the apex of the slender **nucellus**, and close over it, leaving only a narrow opening, the **micropyle**. The closure of the inner integument is first completed, then the outer. The inner integument is uniformly thick over the whole nucellus; the outer is developed only on the free outer side. The nucellus itself is bent in the same manner as the entire ovular rudiment; the ovule is **campylotropous**. In flower-buds more than one mm. high the ovular rudiments have well-nigh completed their development; at the apex of the style the stigmatic papillæ have already begun to form. The stigmas are **commisural**, that is, are placed in a plane corresponding to the partition wall (replum).

Note on the use of Ruthenium red (Ammoniacal Ruthenium sesquichloride) as reagent for Mucilages, etc.

This is a brilliant red dye, which is soluble in water, saturated solution of calcium chloride, and in alum solution, but is insoluble in glycerine, alcohol, oil of cloves, etc., so that preparations stained with it can be preserved in pure glycerine, or glycerine jelly, or can be dehydrated and mounted in Canada balsam. As light precipitates it in the presence of water, the aqueous solution must be kept in a black bottle, or in the dark. Mangin (who introduced it) recommends its use in 0·01 or 0·02 per cent. solution, *i.e.*, one part in 10,000 or in 5,000.

It has the important property of staining pectic compounds, and hence the mucilages and slimes which are derived from them, *e.g.*, the slimes of seeds of *Linum*, *Cydonia*, *Malvaceæ*, and the gums of *Cerasus*, *Amygdalus*, *Prunus*, *Acacia*, *Astragalus*, etc.; but does not stain the slimes derived from cellulose, such as those of orchids, nor cellulose itself. It often stains cuticularised membranes, but not the true cuticle.

It stains the middle lamella, *e.g.*, of wood, and shows up very beautifully the closing membrane of bordered pits, and especially the torus.

In its inertness with cellulose and callose, and active affinity for pectic compounds, the dye compares with basic dyes generally, amongst which, for the purpose of distinguishing these various compounds, it decidedly holds first place (Mangin); and in its preserving qualities it is far superior to methyl blue, naphthalin blue, safranin, etc., which are dissolved out by alcohol.

As far as nitrogenous bodies generally are concerned, its action appears to be unequal; the nuclear chromatin is stained most of all, then leucites, and lastly, but feebly, granular cytoplasm. Its action can be intensified by previous treatment of the preparations with alum solution, but even then it is weaker than many acid and even basic dyes.

CHAPTER XXXII.

CELL-DIVISION AND NUCLEAR DIVISION. FIXING, EMBEDDING, AND MICROTOMY. PROTOPLASMIC INTERCONNECTIONS.

MATERIALS USED.

Young flower-buds of *Tradescantia virginica*, or allied species, June to *September. Fresh.*

Flower-buds of various ages of some Liliaceous plant, such as *Fritillaria persica*, or other species, Tulip, *Lilium*, etc. Fresh. Also the same in absolute alcohol.

Flower-buds, various ages, of a Ranunculaceous (such as *Helleborus foetidus*) or Papaveraceous plant. Fresh.

Old internodes of *Tradescantia virginica*. May to October. Fresh or in alcohol.

Seedlings of Broad Bean (*Vicia Faba*), 2 to 4 days old. Fresh.

Twigs of Mistletoe (*Viscum album*); or *Abies* sp. Fresh.

Seeds of Vegetable Ivory (*Phytelephas macrocarpa*).

REAGENTS USED.

Very various. See text.

A. NUCLEAR DIVISION FROM LIFE.

Cell and Nuclear Division in Tradescantia.—The best living object upon which to follow directly the mitotic or indirect division of the nucleus and the cell, is the staminal hairs of the Spider-wort, *Tradescantia virginica*, which we have already used for the study of protoplasmic movements, or of some nearly allied species. We must observe the hairs, however, in a stage of development in which they are not yet fully formed, but are in active cell-multiplication. For this purpose we take for investigation flower-buds, which, without stalk, measure between $\frac{1}{5}$ and $\frac{1}{4}$ inch in height. We open these buds, and first remove the anthers from the filaments with fine forceps. Then with a scalpel we cut across under the insertion of the ovary and the filaments, and lift this part bodily out of the bud. We lay it in a drop of 3 per cent. solution of sugar, and then set the filaments

free with needles under the simple microscope. The ovary and all the other floral parts are removed from the preparation. We can observe the preparation upon the slide direct, or on a cover-glass placed upside down upon the edges of a moist chamber. In this latter way we can retain the hairs in developmental condition for a half day or more, although those which lie deeper in the suspended drop will not admit of the use of the higher powers. We must take care, therefore, that the suspended drop is spread out flat.

The **resting nucleus** appears finely punctate (Fig. 153, 1, lowest cell); but examined with stronger magnification, or in cells which have been brought somewhat under the influence of the surrounding fluid, we see that the minute granules are not isolated, but closely connected into rows, forming fine convoluted threads; the entire nucleus thus forms a **network** or **framework** enclosed in a delicate **nuclear membrane.** Between the coils of the thread are distinguishable several **nucleoli** of various sizes. The nucleus is surrounded by cytoplasm, which is connected with the peripheral cytoplasm by plasmic threads. The cytoplasm contains, besides the scarcely distinguishable **microsomata** (or **microsomes**), larger, more strongly refractive grains—**leucoplasts.** The nucleus preparing for division increases in size, and from its fine framework forms gradually a coarsely granular thread; it then begins to elongate, and the coils of its thread arrange themselves obliquely, approximately parallel to one another (Fig. 153, 2). At the same time the cytoplasm begins to collect at the two **poles** of the nucleus. We can easily observe all these progressive changes in one and the same cell, but this requires a comparatively long time, and continuous, or only briefly interrupted, observation. The grains in the thread then become indistinct; this assumes gradually a homogeneous aspect, and lays its coils in a definite fashion which is not easy to follow in all its phases. At the same time the nuclear membrane disappears. After a while we can make out with certainty that the nuclear thread has divided into segments, or **chromosomes,** and that these separate from one another so as to produce the distinctive Fig. 153, 3, in which these chromosomes are shown as straight, approximately equal rods, arranged in two groups, of which the equatorial ends are coincident, one with one, and the polar ends are somewhat convergent. Not infrequently the chromosomes have their polar ends bent hookwise. Between the moment when we saw the

threads coarsely granular (Fig. 153, 2) and this stage, over an hour may have elapsed. The chromosomes appear nearly homogeneous, but with a higher power (*e.g.* × 1000) slight indentations of their surface can be recognised, which indicate their composition out of successive discoid segments.

If we have only limited time at our disposal, this stage (Fig.

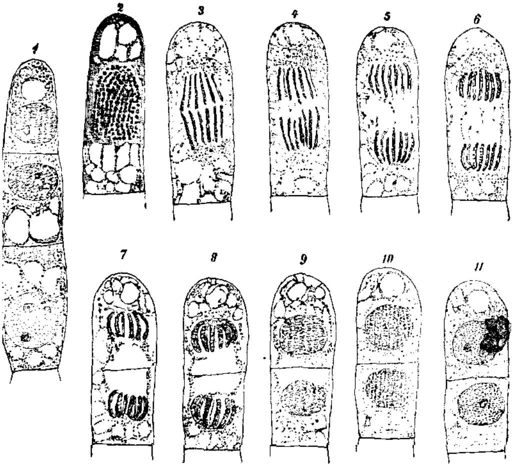

FIG. 153.—*Tradescantia Virginica.* Processes of division in the cells of the staminal hairs. Fig. 1, with a resting nucleus in its lowest cell, and an upper cell which has just divided. Fig. 2, with a coarsely granular nucleus showing oblique striation. Figs. 3-11, successive stages of division followed in the same cell; 3, about 10·10 o'clock; 4, 10·20; 5, 10·25; 6, 10·30; 7, 10·35; 8, 10·40; 9, 10·50; 10, 11·10; 11, 11·30 (× 540).

153, 3) can be selected as that from which to keep the preparation under *continuous observation.* For the next few minutes we await a further separation of the two nuclear halves, *i.e.*, of the two groups of chromosomes; and this takes place then so quickly that it can be closely followed. The two nuclear halves separate from one another in longitudinal direction (4); five minutes later, they

are a noticeable distance apart (5). All the daughter chromosomes do not always separate from one another simultaneously; some may remain behind, and then hasten after the others. At the same time we see the daughter chromosomes, during this movement, bend at the poles, becoming somewhat shorter and correspondingly thicker (5). Between the nuclear halves remains a substance, transparent as glass, which visibly increases in quantity (5 and 6). In this transparent central mass a finer structure is not recognisable, but it is in fact differentiated into threads. It assumes gradually a barrel shape. From twenty-five to thirty minutes may have elapsed since the commencement of the separation, and we see appear in the equatorial plane of the central mass dark dots arranged in rows. They form the so-called **cell-plate.** In the next moment these dots fuse together, and form a sharply defined dark line, a primary membrane separating the two daughter cells. Within this is immediately afterwards developed a young partition wall of cellulose. If the central, barrel-shaped plasmic body has been formed so broad that it fills the entire cross-section of the cell, we see the partition wall at once joined on all sides to the wall of the mother-cell. If, on the other hand, the plasmic mass does not occupy the entire cross-section, it in all cases impinges on one side of the mother-cell; and we see it, after the young partition wall has been formed on this side, move about inside the cell, so as gradually to come into contact in all directions with the wall of the mother-cell, and thus complete those parts of the partition wall which are still wanting (7-9). During these processes we see the daughter chromosomes bend their equatorial ends towards the interior of the nucleus (7, 8). The ends of these chromosomes in this way come ultimately into lateral contact and union. The entire rudimentary daughter nuclei become now separated off from their surroundings by nuclear membranes. The nuclear threads in the daughter nuclei now again begin to become finely granular, and elongate in delicate zigzag coils (see 9, and the upper cell of 1). The coils become longer, produce loops constantly increasing in number; these ultimately anastomose by lateral bridges, and so assume gradually (10 and 11) the condition which formed the starting-point of our observations. At the same time the two **daughter nuclei** increase in size, and we assume that they are nourished at the expense of the surrounding cytoplasm. They approach slowly nearer to the newly-formed partition wall.—About an hour and a half after the

commencement of the separation the formation of the daughter nuclei is complete, and even nucleoli are visible in them (11).

B. NUCLEAR DIVISION BY THE ACETIC METHYL-GREEN METHOD.

It is very rarely that living material can be used, as in the above case of *Tradescantia*, for the purpose of getting an insight into the processes of **karyokinesis** (nuclear division); and even then only the coarser phases are recognisable. A somewhat deeper insight can be obtained by the use of a method which at one and the same operation rapidly kills and fixes the material, and stains the nuclear figures. For this purpose we will make use of acetic methyl-green. While the results are imperfect as compared with those obtained by the more complicated methods described hereafter, they are far more complete than are obtainable from life.

Nuclear figures in Tradescantia.—We will first of all make use of the same material already used, *viz.*, the staminal hairs of *Tradescantia*. We deal with the material in exactly the same way as before, selecting a suitable flower-bud, and immerse the preparation in a large drop of acetic methyl-green which we have already placed upon a micro-slide. We cover it with a fairly large cover-glass, and press gently upon it so as to flatten out the filaments, removing with blotting-paper the surplus of reagent which flows from under the cover-glass. Acetic acid is a very rapid fixing fluid, and though fixation in this case is imperfect, certain nuclear stages stand out much more clearly than in the live condition. We direct our attention especially to the stage in which the barrel-shaped plasmic body, within which cell-division will take place, is recognisable between the rudiments of the two daughter nuclei (Fig. 153, 6). In this barrel-shaped body, which in the living object appears homogeneous, acetic acid fixation enables us to recognise *clearly a fibrous structure: it consists of connecting threads* (**spindle fibres**) running from one to the other of the nuclear rudiments; and shows at the same time that the cell-plate, out of which the primary partition wall will proceed, owes its origin to knot-like thickenings of these spindle fibres in the equator of the cell. To this extent, therefore, acetic methyl-green may usefully supplement studies in living *Tradescantia*.

Root-tip of Vicia Faba.—For another illustration of nuclear and cell-division in vegetative cells, as shown by the acetic methyl-green method, we may take the root-tip of the horse bean, *Vicia Faba*,

Having selected a very strongly growing root, make a number of the thinnest possible longitudinal sections of the apical region, and immerse these at once in a large drop of acetic methyl-green which we have ready upon an object-slide. The resting and dividing nuclei alike are quickly fixed and stained, and a comparison of them with Fig. 153 will enable us to trace out a considerable number of phases of nuclear division, while showing us that the figures our preparation presents are still very imperfect.

Nuclear Division in the Pollen Mother-cells of Liliaceae.—The same acetic methyl-green method is very suitable for the purpose of studying the general character of the processes which take place in the pollen mother-cells of flowers, and thus amplifying in important particulars the work done in Chapter xxviii. Young anthers permit us to obtain comparatively good nuclear figures, by means of which we can easily follow the chief phases of division. Above all favourable for the purpose are the Liliaceae and Amaryllidaceae, since their nuclei are very large; and such genera as *Lilium*, *Fritillaria*, *Alstroemeria*, etc., are mutually interchangeable as objects of study. It is a great advantage to select species in which the flowers grow in many-flowered trusses (*e.g.*, umbels), since each truss will include flower-buds of a series of ages, which open successively. Experiment will show which bud is at the desired developmental stage; hence we test bud after bud, progressively older or progressively younger (preferably the latter), until we find the stage we want. The method is to open a flower-bud, take out an anther with the forceps, place it in a drop of acetic methyl-green' lay a cover-glass upon it, and press it with, say, a needle-holder until the pollen sacs are ruptured and the contents squeezed out. The evacuated pollen mother-cells are immediately fixed and stained. Within certain narrow limits each pollen sac will contain various stages of division; besides which, the developmental state of the various anthers in a bud is not exactly the same; so that the same flower-bud may provide quite a long series of nuclear figures.

In order to form a judgment of these structures it needs to be understood that the nuclear figures of spore, pollen and embryosac mother-cells differ from those of vegetative tissues in several respects. We have already seen (p. 435) that in the preliminaries of division the convolute thread of nuclear ("chromatic") substance breaks up into a number of segments, the ***chromosomes***; and a comparison of cell and cell will show that the number of these

chromosomes in the dividing nuclei of any particular plant is to all intents and purposes constant. When, on the other hand, we examine the mother-cells of the spore-tissue of any given plant we find that, as compared with its vegetative tissues, the number of these segments is reduced to one-half; *i.e.*, there is a certain stage in the development of what will become spore-tissues up to which, at each successive division, the full number of chromosomes is uniformly found, and at, or after, which the resulting daughter nuclei show only half that number. It is this numerical reduction of the chromosomes which marks the stage at which such a tissue ceases to be "vegetative" and becomes "reproductive". So long as the tissues remain vegetative the nuclei in process of division evidence the fact by the uniform number of chromosomes into which the chromatic thread divides up; but in the nuclear segregation which indicates the passage of a vegetative cell into a reproductive cell the chromatic thread divides into only half the typical number of segments, whatever this may be for the plant in question; and this reduced number of chromosomes is maintained in all divisions so long as the cells remain reproductive; while any neighbouring vegetative cells in division show the full number. If this be realised, it is easy to understand that in the processes of fertilisation, when male nucleus coalesces with female nucleus, the two reproductive nuclei, each representing one-half the normal chromatic number, will by their coalescence restore to the resulting conjoint nucleus the full chromatic elements of a vegetative nucleus, (and of dual origin), fully equipped for a period of vegetative development. What really takes place at the time of reduction division, and of which reduction is a morphological sign, is of course unknown; though the natural assumption would be that it represents the reverse process to that which takes place in the coalescence of fertilisation.

Let us now proceed with the flower-bud. If the pollen mother-cells are already divided into four daughter-cells, or the young pollen-grains are already separated from one another, we must go back to younger flower-buds. We can easily recognise, by the thick, colourless walls which the pollen mother-cells have, whether we are looking at them or at young pollen-grains. We go back to successively younger buds until we can see in the nuclei of the, as yet, thin-walled and connected mother-cells a fine threadlike coil. and a flat nucleolus lying against the wall of the nucleus. At this stage of the development the coiled thread contracts under the

influence of the reagent, withdraws (Fig. 154, *a*) from the nuclear wall (this latter remaining uncoloured), and we can determine that this wall is a membrane formed of surrounding cytoplasm. Taking this condition of affairs as a starting-point, it will be possible to follow out in a generalised way many stages of nuclear division, such as are seen, arranged in chronological sequence, in the adjoining Fig. 154. After the first complete division of the pollen mother-cell has taken place, the two daughter nuclei rapidly enter into a **second division,** in which figures arise which differ from those in the first division in not unimportant details (*h*, *i*). As a matter of fact, in this second division in the pollen mother-cells, a longitudinal cleavage of the chromosomes, prior to the separation of the nuclear thread into separate chromosomes, also takes place, but these longitudinally cleft chromosomes do not fold together as in the first division; they are, rather, attached with their equatorial ends to the spindle fibres. In the subsequent separation of the sister chromosomes in the second division, these, because not folded, show a rod-like form (*l*). The first division with folded chromosomes, which in the moment of separation appear **V**-shaped, is distinguished as "heterotypic division," from the ordinary processes of division, which we see, as above, in the second dividing stage. From the sister chromosomes of this second division arise, at the spindle-poles, the granddaughter nuclei, and between these cell-division is completed in the same way as in the first division (*m*). The division of the two sister-cells, *i.e.*, the second division, takes place either in the same plane, or in two planes crossing one another at right angles.

The stages of nuclear division up to and including the longitudinal division of the chromosomes constitute the **prophases,** the separation of the daughter chromosomes the **metaphases,** and the formation of daughter nuclei the **anaphases** of nuclear division. To all intents and purposes the anaphases are the prophases reversed.

Instructive preparations are also quickly obtained by staining alcohol material with fuchsin iodine-green. It is best to prepare a solution of fuchsin and of iodine-green, each in 50 per cent. alcohol, pour the iodine-green into a watch-glass, and slowly add to it the fuchsin solution until the fluid has taken a distinct violet colour. The anther sections to be stained are placed on the object-slide in a drop of this fluid, which, after the lapse of about a minute, is run off by tilting the object-slide, and sucked up with

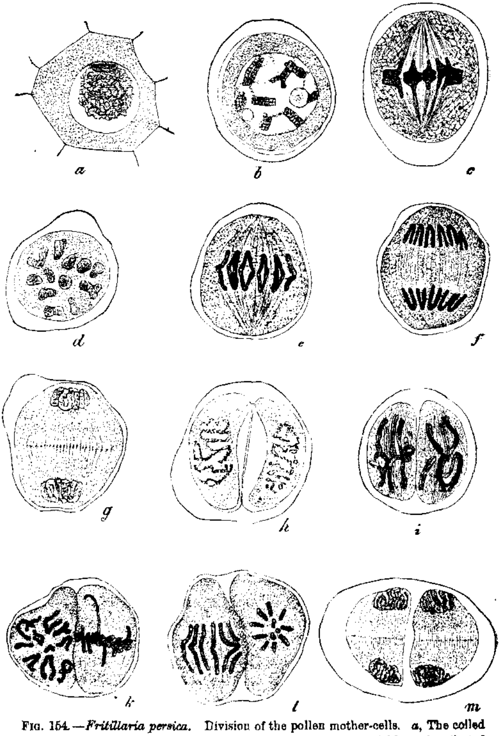

FIG. 154.—*Fritillaria persica.* Division of the pollen mother-cells. *a*, The coiled condition; *b*, the nuclear segments, after longitudinal cleavage and folding, distributed upon the nuclear wall; *c*, the nuclear spindle in profile; *d*, the same as seen from one of the poles; *e*, division of the nuclear plate; *f*, separation of the sister chromosomes; *g*, formation of daughter coils, and of the cell-plate; *h*, opening out of the nuclear threads in the daughter nuclei; *i*, initiation of the nuclear plate; *k*, complete condition of the same and of the nuclear spindle, on the right hand in profile, on the left in polar view; *l*, separation of the sister chromosomes into two segments, on the left in profile, on the right as seen from the pole; *m*, granddaughter coils, and formation of the cell-plate (× 800).

blotting-paper. A drop of glycerine is then placed upon the object, the sections arranged, and covered with a cover-glass. These sections show the cytoplasm red, the nuclear substance blue, the nucleolus stained red; the preparations are exceedingly beautiful and instructive, though inferior in sharpness to those obtained by more complicated methods described hereafter. They can be closed with Canada balsam, and subsequently with gold size. Canada balsam, however, as has been already mentioned (p. 113), is soluble in the oils used for homogeneous immersion; care should therefore be taken not to allow the oil to remain in contact with the balsam, and, after use, to wipe the oil off rapidly. As the Canada balsam used in closing always runs under the cover-glass a little, when it is used the object need not be protected in any other way from the pressure of the cover-glass. If gold size alone is used for closing, it is recommended first to draw two lines of gold size across the object-slide with the camel-hair brush. These lines must be at such a distance that the cover-glass will rest with its two edges upon them. The cover-glass is first laid on when the lines are half dry. The line of gold size drawn round the edge of the cover-glass must be laid on several times, waiting till the previous layer is dry before putting on a new one, and using for the purpose very dilute gold size, diluted with linseed oil. The closure is complete when the preparation held up against the light no longer shows lines of light at the edge of the cover-glass. The object can be protected from the pressure of the cover-glass in the simplest possible way, by laying in the preparation hairs of sufficient thickness, or minute plates of mica. Or, for the protection of objects, before laying the cover-glass upon the object-slide, four spots of wax can be made upon it, by means of the wick of a small wax candle which is temporarily lighted and then put out again. Such wax candles can also be used in order to make a temporary closing layer of wax at the edge of a cover-glass already fixed by spots of wax at the corners.

In *longitudinal sections* through the anthers all the mother-cells are not found in the same stage of division. The stages succeed one another in the one or other direction, which is of considerable advantage to the observer.

Pollen-formation in Dicotyledons.—The same acetic methyl-green method can be adopted in order to obtain generalised information as to the formation of pollen-grains in the anthers of Dicotyledons. For this purpose the flower-buds of most of the small-anthered

Ranunculaceae or Papaveraceae will serve; or flower-buds of the Horse Chestnut do admirably, and with the added advantage that a young truss will show them at many stages. In what follows we will refer to the stinking Hellebore, *Helleborus foetidus*. In a flower-bud which, with stalk, measures from $\frac{1}{3}$ to $\frac{2}{5}$ of an inch in height, we find usually, progressing from within outwards, all the stages of division represented in the successive anthers.

We crush the anthers in acetic methyl-green, and obtain the same figures as in *Liliaceae*, but smaller. After the first dividing stage of the mother-nucleus, a cell-plate is produced in the connecting threads, but again dissolved, while the nuclei prepare for a second division. The figure of the second division exactly resembles the first. The pairs of nuclei are joined by connecting threads. These four uuclei are arranged in the globular mother-cell at the four corners of a tetrahedron (Fig. 155, *B*). Because connecting threads arise free in the cytoplasm in all directions between the four nuclei, it follows, therefore, that to the two systems of connecting threads previously present four more are added. In these six systems cell-plates arise (*B*). These plates are clearly visible; the connecting threads, however, are to be seen only in the most favourable cases. The six cell-plates have the form of quadrants of a circle; they join one another in the interior of the mother-cell. Upon the thick wall of the mother-cell are produced six internal, somewhat projecting, ridges, and to these the cell-plates join with their outer edges. Cellulose walls are quickly formed in the cell-plates, and thus the mother-cell is divided into fonr tetrahedrally arranged daughter-cells (*A*). These four cells soon obtain their own walls, and become free, while the wall of the mother-cell is dissolved.

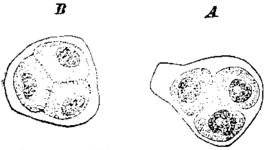

FIG. 155.—*Helleborus fœtidus.* Pollen mother-cells, at *A* in quadripartition; at *B*, after complete quadripartition (× 540). [Three only visible; the fourth is not in focus.]

C. NUCLEAR DIVISION BY MICROTOME METHODS.

For more exact karyokinetic studies the above methods do not suffice. The objects to be studied must be properly selected, well fixed, embedded, sectionised in the microtome, and suitably stained.

How to carry out these processes constitutes one of the most important branches of micro-technics, upon improvements in which recent cytological progress has been dependent.[1]

Fixing and Washing.—The first step consists in properly fixing and hardening the material; the selected material being, as a rule, fixed as promptly as possible, so that the fixing fluid should preferably be taken into the field or garden. The best fluids thus far found for fixing the cell-contents of vegetable cells, though unfortunately expensive, are those known as *Flemming's Fluids*. These are mixtures, in various degrees of strength, of chromic, acetic and osmic acid, the strength depending upon the nature of the part to be fixed, and the extent to which it can be cut up before fixing. The weakest Flemming's fluid[2] contains 0·25 per cent. chromic acid, 0·1 per cent. osmic acid, and 0·1 per cent. acetic acid; a somewhat different but exceedingly efficient weak mixture consists in 60 c.c. of 1 per cent. chromic acid, 8 c.c. of 2 per cent. osmic acid, and 4 c.c. glacial acetic acid, with 72 c.c. distilled water added. The strongest mixture consists of 15 parts by volume of 1 per cent. chromic acid, 4 parts of 2 per cent. osmic acid, and 1 part, or less, glacial acetic acid. The weaker fluid is made use of specially for the smallest objects, and stronger in proportion to their size. As penetration is rather slow it is desirable that, wherever practicable, large objects should be cut up into the smallest possible pieces—*e.g.* $\frac{1}{8}$ inch cubes, or less, or $\frac{1}{8}$ inch slices, or less—before placing in the fixing fluid; and if cutting up is practicable, it is best to use the weakest fluid as above. A large quantity of the fixing fluid should always be used, at least a hundredfold the bulk of the objects to be fixed. With small objects fixing is complete even after a few hours, with somewhat larger ones about a day. After complete fixing, the objects should be

[1] The staminal hairs of *Tradescantia* are not suited to this method of study.— [ED.]

[2] Convenient solutions of these acids for use in the laboratory are 1 per cent. chromic, 1 per cent. acetic, and 2 per cent. osmic, this last being kept in the dark. From these stock solutions any required strength can be made up.

Osmic acid is an admirable fixing reagent, but has what is sometimes the disadvantage of producing a black or brown precipitate with oils, tannic acid, etc. In the present instance this is no disadvantage, but if it were, decolorising of even microtome sections without injury can be effected by means of a solution of 1 part peroxide of hydrogen in 10 to 25 parts of 70-80 per cent. alcohol. Xylol, creosote, etc., if used in the mounting processes, may also decolorise them.—[ED.]

carefully washed in running water, so that all the fixing fluid is removed from them. For this purpose a glass crystallising dish with vertical walls may be used with success. This is covered with a sufficiently close-fitting glass disk, perforated at its centre by a circular hole. Through this central opening a thin stream of water must be conducted from the tap, and the overflow water passes through the same hole.[1] The inflowing water washes the objects about freely, but in spite of their energetic bath they do not leave the sides of the dish.

Another fixing fluid, which usually answers very well, and penetrates the objects with comparative ease, besides having the advantage of being much cheaper, is *Carnoy's acetic alcohol*, a mixture of 3 volumes absolute alcohol and 1 volume glacial acetic acid. For root-tips of *Tradescantia* Farmer and Shove recommend a modification of this, consisting in 2 parts absolute alcohol and 1 part glacial acetic acid. The mixture is allowed to act for from fifteen to twenty minutes, and the acid is then washed out (as judged by smell) with absolute alcohol,[2] and the material embedded as soon as possible. Another good fixing fluid recently introduced by Juel is a mixture of 2 gr. zinc chloride, 2 c.c. glacial acetic acid, and 100 c.c. of 45-50 per cent. alcohol; in which the objects have to remain for about twenty-four hours. In this case washing will be carried on with 45-50 per cent. alcohol, and dehydrating effected by means of higher strengths.

Dehydrating and Embedding.—As an embedding medium, paraffin is best; but before it can be used, the material must be deprived of water up to the last trace (dehydrated). This is done, *gradually*, with alcohol. After the fixing fluid has been completely removed by "washing," the material may be placed in alcohol of 50 per cent. strength, unless, indeed, the washing has itself been effected by alcohol of higher strengths than this, as in the acetic alcohol methods of fixation. After two hours in 50 per cent. alcohol, the material is transferred for the same length of

[1] In a somewhat similar appliance I have found it convenient to slip a couple of india-rubber bands over disk and cover, close on each side of the central hole, whereby the cover is kept in place quite securely. This method is not so safe with filamentous algæ.—[Ed.]

[2] For economy, a preliminary washing with strongest methylated spirit may be made. The utility of these mixtures is due to the extreme rapidity with which both acetic acid and alcohol penetrate the tissues, and the fact that the shrinkage due to alcohol is compensated for by swelling due to acetic acid.—[Ed.]

time successively to 70 per cent., 80 per cent. and 95 per cent. alcohol. In this last the material can be preserved, if need be. Duty-free alcohol is now generally obtainable for English laboratories; but where this is not the case, or with private workers, economy may be sought by using methylated spirit up to the 80 per cent. strength, or thereabouts, for the preparation of which a table will be found in Appendix III. For the higher strengths, absolute alcohol is essential, although the material may, with economy, be first placed in strongest methylated spirit. There is, of course, no special virtue in the exact grades of strength indicated; but it is essential that the process of dehydration should be gradual. If we are dealing with very small objects, it is desirable, in order to avoid losing them, to fix, wash and dehydrate in Fairchild's "perforated pail" (Fig. 156), which can be obtained from Gerhardt's at Bonn, price from 4d. to 6d. It is a small perforated receptacle of unglazed porcelain, in which the object can remain while the fixing fluids, etc., act through the perforations.

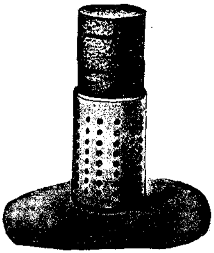

FIG. 156.—Fairchild's perforated pail for small objects, closed with a cork.

It is perhaps even better to use for fixing, washing and dehydrating small glass cylinders, with ground-in ball-topped stoppers, of a kind which are used for museum purposes, the most suitable sizes being 3 to 8 c. high and 3·5 c. broad. After the object has been fixed in such a cylinder, using it as a bottle, the stopper is removed, and the mouth of the cylinder covered with fine muslin or gauze, which can be gummed and tied on. The cylinder can then be placed in a vessel with flowing water. After washing, the cylinder, without opening, can be transferred to the dehydrating fluid without shifting the object.

Another very effective way of carrying out all the processes without rehandling the material is [ED.] to use the double-bowled spoons which have come into use in recent years as tea-infusers. These can be obtained electro-plated, and with perforations no larger than very fine pin-holes (H. J. Cooper's patent, "Unicus," No. 2).

Out of the 96 per cent. alcohol the objects must be laid for an hour or two in absolute alcohol, and care must be taken that this alcohol is to all intents and purposes free of water. This can be best secured by adding to commercial absolute alcohol some sulphate of copper which has been thoroughly dehydrated anew by calcining.[1] The object is thence transferred to a mixture of half absolute alcohol, half chloroform, in which it will at first float, but must ultimately sink. In some cases this may take a fairly long time, but then, and not till then, we may transfer it to pure chloroform. After complete saturation in this, that is for pieces of tissue in not less than twenty-four hours, the object is placed in a glass box or a porcelain basin with chloroform to which a few shreds of paraffin of melting-point 45° C. have been added. This vessel is placed in a warm chamber, paraffin oven or water bath, heated to about 55° C., and left there for one or two days, or, if the object is not very permeable, for even longer. From this solution the object is placed in pure melted paraffin of 45° C. melting-point, remaining therein in a warm chamber for about a day, then transferred to paraffin of 52° C. melting-point for one or two days —in all cases long enough to be completely saturated. The fluid paraffin and the object are now turned into a sufficiently small vessel, for example, a small "box" or trough made of folded writing-paper, or a watch-glass which has been previously smeared with glycerine.[2] An attempt is made to place the object in the most suitable position, or, if there are a number, to distribute them in the paraffin, and this is then rapidly solidified. This can be effected by plunging the small vessel into cold water. If watch-glasses are used for the purpose, they should first be floated on the cold water, and only immersed when the surface of the paraffin has set. Smearing the watch-glass with glycerine enables the hardened paraffin to be easily separated from it. It is desirable always to keep paraffin of 52° C. melting-point in a glass beaker in the warm chamber, and thus at all times to have it fluid for use; and one can then use just so much as is necessary to produce a proper distribution of the objects in the watch-glass. A watch-glass can even be filled with such paraffin, and the objects taken singly out of the paraffin in which they were impregnated, and at once

[1] I find it convenient to enclose this in a piece of glass tubing, plugged at each end with cotton-wool.—[Ed.]

[2] The small porcelain troughs used for moist water-colour paints, well smeared with glycerine, are excellent for this purpose.—[Ed.]

suitably arranged in this new quantity of paraffin. We then cut the paraffin into small cubes or rectangular prisms. The paraffin cubes or prisms prepared in this way are fused to a large block of paraffin by means of a bot needle. This large block will be of such size as lends itself to ready fixing in the object holder of the microtome, and is high enough to enable suitable sections to be cut. The microtome razor must be fairly parallel to a side of the angular paraffin block in which the object is contained. If, however, as with such embedded preparations only quite exceptionally happens, we cut, not with a cross but an obliquely-fixed razor, then the paraffin containing the preparation should be cut in the form of a three-sided prism, and care taken that the side next the razor should be arranged parallel to it.

The transfer from alcohol to paraffin through the intermediary of chloroform has been proved best for our purposes ; but on the other hand cedar oil has heen specially recommended, and has the advantage of great simplicity. For usual use we take not the thickened oil needed for an immersion fluid for homogeneous objectives, but the ordinary cedar oil often quoted in price lists as "for clearing ". Some cedar oil is put into a small cylinder, and absolute alcohol floated carefully upon it. The object is now transferred from absolute alcohol into that which forms a layer over the cedar oil, and slowly sinks through it into this latter. The alcohol is then carefully poured or pipetted off again from the cedar oil, and the object either transferred for balf an hour into half-and-half cedar oil and paraffin, and thence into pure paraffin, or else laid at once in pure paraffin. Most objects admit of this simplification in treatment, and even the transfer at once into paraffin of the higher melting-point. In this they will be already impregnated after about three hours, and after cooling will he ready to cut.

Any double-walled warm chamber, such as is used as a drying chamber, or for culture experiments, can be used as a paraffin oven. The space between the two walls is filled with water, into which, or into the oven, a "thermo-regulator" or "thermostat" projects, which regulates the passage of gas to the flame found under the apparatus. Such ovens are made by many scientific apparatus makers.

A very reliable double-walled chamber, by Jung of Heidelberg, which is equally valid as a drying oven or for use with paraffin, is illustrated in the adjoining Fig. 157. It is made of sheet

copper, 26 c. long, 18 c. deep, 24 c. high, and can be hung upon a wall by means of the iron band and ring. It is provided with a glass door, gas tubes (*a*, *b*) and a nickel-plated micro-burner, with mica chimney (*L*). The space between the double walls is filled with water. The roof of the oven has three openings; in that farthest to the left a thermometer (*t*) is inserted, which dips into the water between the walls, and gives its temperature.

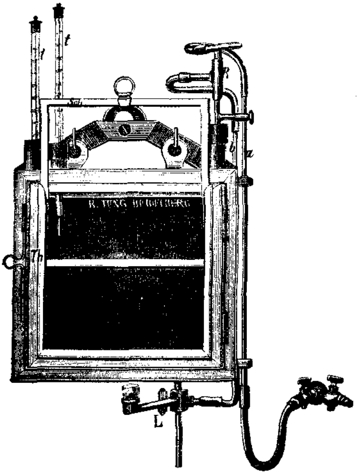

FIG. 157.—Paraffin oven and warm chamber, Jung 's Heidelberg model, No. 292. For description, see text.

Another thermometer (*t*) passes clear through the roof into the chamber itself, and gives the temperature there; but for our purposes this is not necessary, the temperature of the water jacket sufficing, while the thermometer itself occupies valuable space, and is liable to be broken. We will therefore do without this second thermometer, closing the aperture with a well-fitting cork. Into a third opening is placed an automatic thermo-regulator (*R*), the mercury bulb of which is immersed in the water jacket. The gas

passes from the supply pipe through the hollow stopper of this thermo-regulator, and then, in the case illustrated (Reichert's), passes out in a fine continuous supply through a pin-hole in the side of this stopper, and a much larger intermittent supply through the open bottom end of the stopper, this intermittent supply depending upon whether the mercury in the thermostat expands sufficiently, with heat, to cut the current off, or contracts sufficiently, with cooling, to let it pass; the resulting supply passing thence to the burner by means of the passage tube projecting horizontally opposite to *B* in the figure. When the lower, and main, supply from the thermo-regulator is cut off, that which passes permanently through the pin-hole (as a "by-pass") suffices to keep the flame alight. By means of a screw working in another horizontal arm to the thermo-regulator (shown above the letter *a*) mercury can be displaced in the thermo-regulator so as to open or close the lower gas-opening at any desired temperature, as seen by the thermometer *t*; and thus the main gas supply can be kept automatically regulated at that temperature. If the chamber rises above the given temperature, the main gas supply is cut off by the expanded mercury closing the aperture, and the flame goes down; should the temperature fall the opening is again freed, more gas passes, and the flame goes up. This kind of thermo-regulator is very sensitive.[1]

With an obliquely-placed razor only single sections can be taken; with a straight razor a series of successive paraffin sections can be cut, as the paraffin sheets will come into contact, and cling by their edges into a continuous riband. If the razor is properly arranged and sharp, the sections should not be pressed together, that is, they should preserve approximately the same cross-section which the paraffin cube itself shows. Should the sections curl up, this arises either from the too great hardness of the paraffin, or the too great thickness of the sections, or the two combined. In general the riband slides readily from the razor, or if any slight disturbance arises, the band may be eased by a curved needle (such as Fig. 158, *B*), or a semi-lancet-shaped needle (Fig. 158, *A*). The curved needle also serves to remove the riband, which can then be cut into pieces of suitable length by means of a very narrow scalpel (Fig. 158, *C*). It is very desirable to provide large cover-glasses,[2] so that the band need not be cut into too

[1] I use heavy machine oil as a jacket instead of water.—[Ed.]

[2] Size $1\frac{1}{2} \times \frac{1}{2}$ inch.

short sections. If the band should be somewhat crumpled, it can be laid on the back of the hand, and drawn with the needle towards one end. The warmth of the hand and the slight resistance which it offers facilitate the straightening of the band.

It is now necessary to fasten the paraffin sections, or series of sections, upon the object-slide. We can do this either with water,

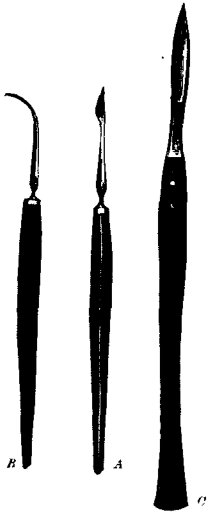

FIG. 158.—*A*, semi-lancet; *B*, curved needle; *C*, small scalpel.

or with glycerine albumen, or with a combination of both methods. Pure dust-free water is slightly warmed in a shallow porcelain dish, and the sections placed upon this with the needle. They spread out on this water very beautifully, and are lifted out of it by the object-slide being pushed underneath. They are then allowed to dry in the air, and laid for about an hour or longer on a shelf in a paraffin oven or else in a warm chamber such as

that figured upon page 450. The paraffin is afterwards dissolved in turpentine or xylol, while the sections of the object remain adhering to the object-slide. If fixing to the object-slide with glycerine albumen is preferred, a mixture of equal parts egg-albumen and glycerine is used, the solution being filtered. If a piece of camphor is put in, it will keep for some weeks.[1] It is placed on the object-slide in only very small quantity, and rubbed smooth with the ball of the hand, forming thus an unrecognisable layer. The sections are now laid on it, and pressed with a dry camel-hair brush, or the dry finger, or a quite dust-free piece of linen. The object-slide is then warmed over a flame till the paraffin begins to melt, and this latter is then removed, just as in the other case, with turpentine or xylol. The combination of the two methods consists in rubbing the glycerine-albumen on the object-slide, and then pouring on a little warm distilled water. On this water, which may again be slightly warmed, the sections are floated and arranged, and after they are properly spread out, the water taken up with blotting-paper, the preparation dried in the air and warmed over a flame. It has also been advised, after rubbing on the glycerine-albumen, to coagulate the albumen in the warm chamber at about 70° C., then pour on the water, transfer the sections, and warm the water slightly until the sections have spread out quite smoothly. Then remove the water with blotting-paper, and dry the preparation in air or in a warm chamber at about 30° to 35° C. Similarly pure albumen has been used. This is shaken up for a considerable time with ten times its bulk of water, filtered, spread out on the object-slide by means of a glass rod, and allowed to dry in a vertical position. The microtome sections are laid on water at 40° C. in a large dish, a prepared object-slide pushed flat under them, and the sections lifted out. They are then dried at 35° C., the paraffin removed with xylol, and this latter with absolute alcohol.

The Compound Microtome.—While, until recently, microscopic sections were almost always cut with the hand, and of microtomes, at any rate, only the simple hand microtomes were in use, now the compound microtome is found in all botanical laboratories. Every one, therefore, who wishes to work with success in the higher reaches of histology must now acquire a knowledge of its manipulation. The number of good microtomes manufactured by

[1] Or a few drops of carbolic acid can be added before filtering.

various mechanicians is now very large. Most of them have their partisans, and most workers tend to declare that microtome the best to which they themselves are accustomed. In construction the different microtomes which are now in use are distinguished from one another in that with some the razor and the object to be cut move on approximately horizontal slides, in the others there is a horizontal carrier for the razor and a vertical one for the object, and with still others the razor is fixed and the object is carried against it.

For some years we have used the Thoma-Jung sliding microtome,[1] and have found that it has thus far answered all the demands which we have made upon it. These microtomes do not work so rapidly as many others, but in respect of certainty of action, and the uniformity in thickness of successive sections, can scarcely be surpassed. Owing to the complicated structure and difficulties in use of this and other large microtomes, we will confine ourselves to a single pattern, issued at a not too high price; premising, however, that there are others which work equally well, and some possibly even more perfectly. It is naturally best to learn the art of manipulating a microtome under the practical guidance of a teacher, as mere descriptions are a very poor aid.

The Rocking Microtome.—The microtome most used in Great Britain is perhaps the new Rocking Microtome, made by the Cambridge Scientific Instrument Company, which is illustrated in the adjoining Fig. 159, where its construction is readily recognisable. Upon a massive base plate are two strong uprights, made in the same casting; that upon the left hand serving to carry, by means of clamps, the blade of an ordinary razor, while that rather more to the right serves as a fulcrum for a lever with a long horizontal arm and a short vertical arm. The end of the horizontal arm is moved upwards by a screw; thus the upper end of the short vertical arm is moved towards the razor, but by a less amount than the vertical movement given by the screw. The upper end of the vertical arm of the lever forms the fulcrum of the rocking arm which carries the object to be cut. By means of the handle at the extreme right an adjustable pawl is actuated which engages the teeth of the milled head of the vertical screw, the amount of movement, and consequently the thickness of the section, depending upon the

[1] The Thoma-Jung microtomes can be obtained through W. & J. George, Limited (successors to Becker & Co.), London and Birmingham.

number of teeth the pawl had been adjusted to miss. The rocking arm carries the object embedded in its paraffin block, fused on to a permanent paraffin block contained in a tube clamped to the rocking arm itself; and the same movement which, in its last stage, actuates the pawl also lowers the rocking arm by means of a cord, and carries its paraffin end upwards past the razor; while in its recovery, brought about by a strong spring, the object to be cut is thrust against the razor edge, and the section is cut. The thickness of the sections is easily adjusted, and is read by a pointer and graduated arc; each division on the arc is $\frac{2}{1000}$ mm.

In the latest pattern the object in its upward movement cannot touch the razor, as by means of a simple device it is drawn backwards before the upward movement begins. By this means

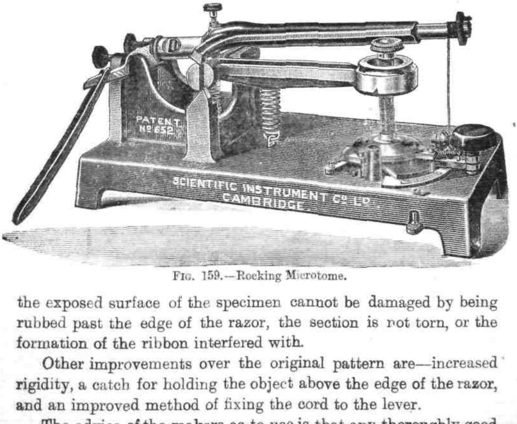

FIG. 159.—Rocking Microtome.

the exposed surface of the specimen cannot be damaged by being rubbed past the edge of the razor, the section is not torn, or the formation of the ribbon interfered with.

Other improvements over the original pattern are—increased rigidity, a catch for holding the object above the edge of the razor, and an improved method of fixing the cord to the lever.

The advice of the makers as to use is that any thoroughly good razor can be used, and the object to be cut should be embedded in hard paraffin, melting at about 55° C. The block containing the object should then be fixed to the paraffin contained in the object-holder of the microtome, by gently melting the surfaces. The sides of the block must now be cut so that the opposite sides are parallel; it is then dipped into melted soft paraffin, that is,

paraffin with a low melting-point (about 48° C.), so that it is coated all over, the object of which is to secure the cohesion of the successive sections. The coating of soft paraffin is then removed from two opposite sides, and if necessary the thickness is reduced on the other two sides. Unless the sides are flat and the coating of soft paraffin is very thin, the sections will not form a good ribbon. The object-holder with the block attached is now ready to be replaced on the microtome, care being taken that the coated sides of the block are above and below, so that one of the coated sides shall first come into contact with the edge of the razor. The microtome will now cut sections adhering together and forming a ribbon. The largest section which can be cut is 20 mm. in diameter.

In spite of its comparatively low price (£3 15s.[1]), the Rocking Microtome makes beautiful series of sections. A disadvantage which it has in comparison with some microtomes is that, inasmuch as the rocking arm moves through an arc, the section cut is not a true plane but a portion of a cylindrical sheet, and therefore is, even if only slightly, curved. For most small histological objects this, however, does not matter, and hence its wide use in botanical laboratories.

Material embedded in paraffin is usually cut in series of sections, with razor set square, its edge acting chisel-wise. The quality of the cutting edge is therefore of the highest importance, if very thin sections are to be cut with regularity. While any "thoroughly good" razor can be used, the ordinary sectionising razors used for hand work are quite unreliable, and it is better to have razors, which may be quite short blades only, for this special purpose. Those of Henking[2] are very highly recommended. They are made in two thicknesses, *a* and *b*.

Microtome and razors must be kept scrupulously clean. The working parts of the former should be oiled with best jeweller's oil.

Staining Microtome Sections.—The staining method which has hitherto proved best for the cell-contents of vegetable objects in

[1] With a razor and simple orientating object-holder, by means of which the material to be cut can be adjusted in respect to the razor, the price is £4 2s. 6d.

[2] Henking's razors can be obtained through any good instrument firm or direct from Wilhelm Walb, Heidelberg, Hauptstrasse 5, No. 16 in his list, at M. 6·50; a sheath to contain two razors is M. 2·50.

microtome sections adhering to an object-slide is Flemming's triple staining with safranin-gentian-violet-orange, commonly known as Flemming's orange method. Three colours come into use in it, this being the greatest number which hitherto have, with any advantage, been combined in the same object. These colours are not mixed, but used consecutively, and each of them requires a definite duration of action, and suitable subsequent treatment. Objects which have been fixed with Flemming's solution, or other similar fluids containing chromic acid, always give very good results with this staining; but with alcohol material, on the other hand, only after the sections fixed on the object-slide have first been soaked in 1 per cent. chromic acid. The object-slide with the sections is for this purpose left for twenty-four hours in the chromic acid, then transferred to pure water and there left to wash for two hours. Under no circumstances, however, will the staining proceed unless all the paraffin has been removed from the section by washing in turpentine and afterwards in absolute alcohol.

The objects must be laid for a day, in some cases longer,[1] in a dark alcoholic solution of safranin. For this purpose one uses a concentrated solution of safranin in alcohol, to which an equal volume of water and a little aniline water have been added. The sections are then treated with alcohol, and then with alcohol to which 0·1 per cent. hydrochloric acid is added, and then again washed with pure alcohol. The washing, especially with acid, must be careful, in order that too much safranin be not removed from the sections. In general the needful degree of decolorising is very quickly attained. The object-slide is then placed for about three to five minutes, under some circumstances, however, much longer, in a dark (concentrated) watery solution of gentian-violet. It is then rapidly washed in water, or can be transferred direct to a dark (concentrated) watery solution of orange G.[2] which is allowed to act for one or at most two minutes. The sections are again washed in absolute alcohol, and the washing continued in oil of cloves for so long as blue clouds of gentian colour come out of the section, when it is finally mounted in Canada balsam dissolved in xylol, and covered with a cover-glass. Care must be taken that not more Canada balsam is placed under the cover-glass than will occupy the given space. That only thin cover-glasses must be used goes without saying, bearing in mind the high powers we

[1] Flemming recommends two or three days.—[Ed.]
[2] One of Grübler's stains.

shall wish to examine the preparation with; and it is well to obtain extra large cover-glasses whereby proportionately longer series of sections may be covered.

The critical matter in this method of staining is to determine the exact point at which staining or decolorising should be stopped. Hence the length of action of the separate stains, and of their subsequent washings out, must be found by experiment for each object. It differs for each according to the nature of the object and the method of fixing. While, for example, for staining the contents of certain pollen mother-cells in process of division the duration recommended above for staining, washing, dehydrating and clearing holds good; on the other hand, with sections through the oogonia of *Fucus* we have obtained the best results with safranin staining for only half an hour, gentian violet ten minutes, and orange G. staining for one minute. Staining in orange G. must follow washing in water, and cannot come directly after treatment with alcohol. If absolute alcohol is used at this stage the complete removal of the gentian-violet will result.—If this triple staining has been successful, the different constituents of the protoplasm are stained in different fashions, thus materially aiding their differentiation.—As vessels for staining, small beakers can be used, each of suitable size and depth to take one object-slide, and this independent treatment of each object-slide has advantages. [Or a number of slides can be stained together by placing one glass crystallising dish within another of suitable size, weighting down the inner one, *e.g.*, with shot or sand, and filling the space between the two with the staining fluid. It is safest to place the slides section-face outwards. If the crystallising dishes have lips, these can serve as starting-points in the placing and recognition of the several object-slides. Several such crystallising dishes can be placed within one another, the central one only being weighted. This method also economises the staining material.]

In purchasing stains it is of great importance to get those which work correctly, and hence to obtain them from a firm who are acquainted with the scientific needs of microtechnics.[1] The best micro-stains are those prepared by Dr. G. Grübler & Co. of Leipzig.

Special advantage is often derived from staining with Heidenhain's iron alum-hæmatoxylin, the method used being as follows:

[1] Messrs. Southall Bros. & Barclay, Manufacturing Chemists and Scientific Instrument Makers of Birmingham, have undertaken to keep Grübler's stains.

After the sections have been freed from paraffin by means of xylol, and of xylol by use of absolute alcohol, and the darkening due to osmic acid has been reduced by a longer or shorter stay in turpentine, and this last again removed by absolute alcohol, the sections are transferred for a few minutes to 95 per cent. alcohol, and thence to distilled water, in which they are washed. The sections are then laid in a 2 per cent. watery solution of Heidenhain's ammonium sulphate of iron (one of Grübler's reagents), in which they can remain for a night, or up to twenty-four hours. This solution does not stain them, but acts as a mordant to the staining solution of hæmatoxylin. Now wash the sections in distilled water and place them for twenty-four hours in a fairly old solution of hæmatoxylin made with $\frac{1}{2}$ per cent. in 5 parts absolute alcohol to 100 parts water. After the action of the logwood the sections will appear deeply stained. They must now be thoroughly washed in tap-water, and for the purpose of differentiation again placed in the 2 per cent. watery iron alum solution, which will remove a large part of the stain. This process of differentiation by decolorising had best be controlled under the microscope, and stopped when the chromosomes appear sharply defined; the process being then stopped by transfer to running tap-water, in which the slide can remain for a quarter of an hour or more. After this washing, dehydrate in 95 per cent. alcohol, then in absolute, then pass through xylol and mount in xylol Canada balsam.

Nuclear Studies in Root-tips.—The apices of roots are specially suited for practice in micro-technics, and for the study at the same time of nuclear and cell-division. The horse-bean, *Vicia Faba,* is very suitable, since material can be obtained at any time in the year. It suffices to lay the beans for a few hours in warm water, then set them in damp sawdust in a warm place. They germinate at once, and in from two to four days the roots will be ready to fix. For this purpose the second of the two weak Flemming's fluids mentioned on page 445 should be chosen, the tips of the roots being cut off at not more than 6 mm. from the apices. After the manipulation described above is complete, the root-tips should be so embedded in paraffin as to offer themselves for the truest possible longitudinal sectionising. In the preparations when ready it will not take long to find nuclear and cell-divisions. These offer themselves essentially as in the somewhat diagrammatised arrangement of Fig. 160, which shows the sequence of events.

The figure shows as in 1 a nucleus still resting, provided with

one or more nucleoli (*n*), from the fine network of which the thicker and shorter nuclear thread (*ch*) of 2 is separated out. At the same time the chromatic contents, and with it also the capacity for staining, increase; yet it can be determined that it is only the consecutive **chromatin disks** of the thread which show strong staining powers, and that they are connected together by bridges of unstained **linin**. From this arises the cross-striation of the nuclear thread which shows more or less clearly in many places. After this the nuclear thread falls into a definite number of segments, the **chromosomes** (3 and 4). These now approach towards the plane of division, in order to form the so-called **nuclear plate** or equatorial plate (5); but before this the nucleoli and the nucleolar membrane have disappeared. Each chromosome has undergone longitudinal cleavage (6), though this is only recognisable with the most successful fixing and staining. The two longitudinal halves of each chromosome, the **daughter chromosomes**, now separate from one another in opposite directions, so as to form the rudiments of the daughter nuclei (7-9). While all these processes take place in the nuclear thread, other differentiations in the cell-body become recognisable. At the time when the nuclear thread becomes shortened, disentangles itself, and falls into the separate chromosomes, cytoplasmic threads invest the nuclear membrane, and surround it with a fibrous sheath. With favourable action of the three stains we have used, this fibrous layer will show a violet coloration, while the rest of the cytoplasm is brown. These fibrillæ represent a particularly active constituent of the cytoplasm, which may be distinguished as **kinoplasm**, and stand out prominently in the processes of division alike of the nucleus and of the general cell-body. The fibrous layer quickly becomes raised from two opposing sides of the nuclear wall (2, *w*), and thus forms the **polar caps** (2, *p*). Homogeneous, delicate fibrillæ become recognisable in these which stream pole-wards. The polar caps elongate and become pointed, whereby their fibrillæ become arranged into two converging polar bundles (3). While the nucleolus now becomes dissolved, and the nuclear membrane disappears, the fibrillæ of the polar caps become prolonged into the nuclear cavity (4). Some now attach themselves to the chromosomes, while of the others the equatorial ends come into union, so that they run as uninterrupted threads from one pole to the other. In this way arises the **nuclear spindle** (4). Those spindle fibres which attach themselves to the chromosomes are traction fibres, those running from pole to pole are supporting fibres. The traction

fibres, after they have taken hold of the chromosomes, bring these into the equatorial plane (5, 6). The separation of the daughter chromosomes, which arise from the longitudinal splitting of the mother chromosomes (7, 8), may be due to the contraction of the traction fibres. The daughter chromosomes are thus assisted in the direction of the spindle poles. The supporting fibres give the necessary resistance for this process. In the rudimentary daughter nuclei the free ends of the chromosomes soon become bent inwards (9),

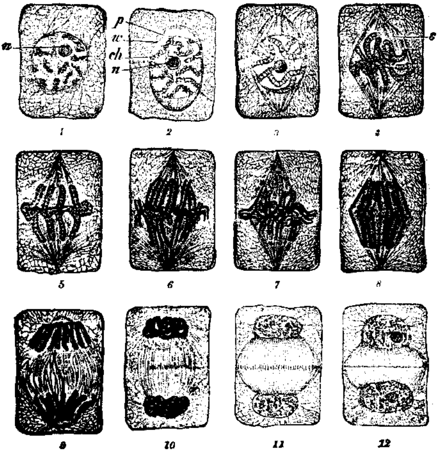

FIG. 160.—Successive stages in nuclear and cell-division in an embryonic tissue in the neighbourhood of a growing point. (Somewhat diagrammatic.) *n*, nucleolus; *p*, polar caps; *w*, nuclear membrane; *ch*, nuclear thread; *s*, spindle fibres (× about 600).

and the surrounding cytoplasm forms a limiting layer towards the rudimentary nucleus and forms the nuclear membrane (11). Inside the rudimentary nucleus, the chromosomes become joined into a network, the chromosomes being unrecognisable, though probably retaining their individuality; the nuclear rudiment grows larger, one or more nucleoli appear, while the extra-nuclear muleoli disappear in the cytoplasm; and the daughter nucleus now enters upon a resting phase (*c.f.* fig. 161).

Specialised centres of attraction (centrosomes) at the poles of the nuclear spindle have not been proved to exist in the higher plants; they are, however, universal in animals, and are present in the lower members of the vegetable kingdom.

Our preparation of *Vicia Faba* enables us also to readily follow the progress of cell-division, which, in the higher plants, is associated with nuclear division. Between the separating chromosomes, the supporting fibres, extending from pole to pole, continue as connecting fibres (8, 9). By the interpolation of new connecting fibres their number is increased, especially in the neighbourhood of the equator (10). They ultimately form a barrel-shaped body, the threads of which soon swell rodwise in the equatorial plane, so as to form the **cell-plate** (11). This extends on all sides to the mother-cell wall, so that the primary membrane which arises from the cell-plate divides the mother-cell at once into two daughter-cells (12). Immediately afterwards the division wall of cellulose is formed within, and splitting, the primary membrane.

For comparison with Fig. 154, in which the karyokinetic figures in pollen mother-cells of *Fritillaria* are illustrated, as shown by the acetic methyl-green method, we introduce here, in Fig. 161, similar but more complete figures derived from *Lilium Martagon*, as displayed by the methods just described, that is fixing with chromosmium-acetic acid, embedding in paraffin, sectionising with the microtome, and staining with the triple stain.

All the processes of nuclear division which show an internal thread-like differentiation are collected together under the term **indirect or mitotic division**, as opposed to the **direct division**, which consists in a simple constriction of the nucleus. Such direct nuclear division is often found in the older cells of higher plants, and as an exceptional case in the actively growing internodal cells of the Characeæ (Stoneworts).

Direct Nuclear Division.—For the observation of **direct or amitotic nuclear division** in older cells the older internodes of *Tradescantia virginica* are especially suited. A longitudinal section, examined in water, shows them usually in considerable number (Fig. 162, *A*). The nuclei show the same internal structure as before, but are, however, more or less irregularly constricted into sections of various size and form. If the constriction is unilateral, the nucleus appears kidney-shaped; with constriction all

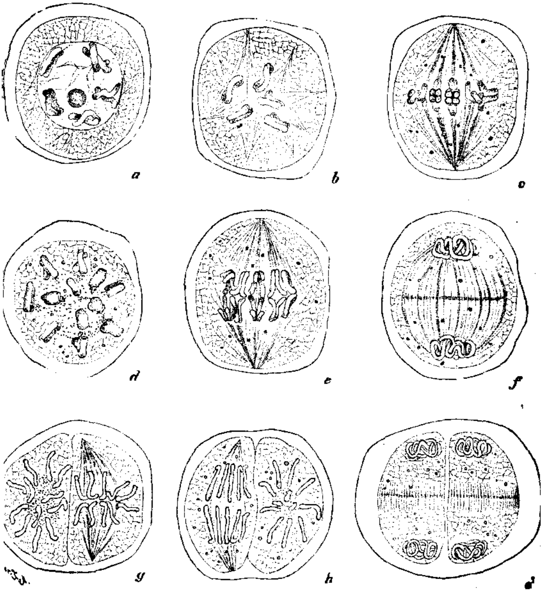

FIG. 161.—*Lilium Martagon.* Division of the pollen mother-cells. *a* to *f*, first division; *g* to *i*, second division. *a*, the chromosome-pairs distributed over the nuclear membrane, between them the rounded nucleolus; *b*, multipolar origin of the nuclear spindle; *c*, complete bipolar nuclear spindle, the chromosome-pairs attached to the spindle fibres as an equatorial nuclear plate; *d*, the nuclear plate in polar view; *e*, separation of the two chromosomes of each pair, and formation of V-shaped figures by the longitudinal splitting of the daughter chromosomes; *f*, rudiments of the daughter nuclei, and formation of the cell-plate in the connecting fibres; *g*, nuclear spindles and nuclear plates of the daughter-cells as seen on the right in lateral, on the left in polar, view; *h*, separation of the daughter chromosomes, as seen on the right in polar, on the left in lateral, view; *i*, rudiments of the granddaughter nuclei and of the cell-plates in the connecting fibres. The grains distributed in the cytoplasm in *c*, *d*, *e*, *f*, *h*, and *i* are extra-nuclear nuclei, derived probably from surplus nucleolular material. The figures will show that the two rapidly successive divisions of the nucleus to form the four pollen grains are somewhat unlike, and also unlike what is to be seen in other tissues. The first of the two divisions has been called heterotypic, the second homeotypic, while both can be contrasted with the typical division of vegetative tissues as atypic (× 750).

around, it shows as a figure of 8, or perhaps irregularly lobed. In many cases the segments have completely separated, and lie either in contact or more or less remote. The number of the nuclear segments thus found in one cell can amount to eight or ten. They are of various sizes. The nuclei in course of constriction are to be found in almost all the elements in the section, most easily in the parenchyma of the central cylinder. The nuclei can be fixed very quickly with acetic methyl-green (Fig. 162, *B*), and are then very sharply defined.

Intercellular Connection of Protoplasts.—As a last study we will use our most powerful objective in order to obtain insight into a

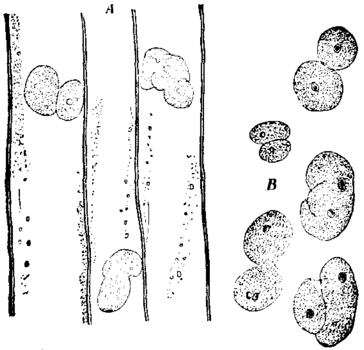

FIG. 162.—*Tradescantia virginica.* Nuclei of older internodes in direct division. *A*, during life; *B*, after treatment with acetic methyl-green (× 540).

relationship the determination of which is of the highest importance to a collective conception of the plant body; we mean the union of protoplasts by means of plasmic threads, whereby multicellular organisms of the vegetable kingdom also can become co-ordinated into connected living entities.

The Mistletoe (*Viscum album*) provides the most suitable object for this investigation; the method consisting in fixing the cell-contents, swelling the cell-walls, and staining the plasmic threads. For the purpose we select internodes which are quite fully developed, yet not too old, and prepare surface sections. Commencing with the epidermis, we take a few consecutive sections from the same

spot; these will consist chiefly of primary cortex. The moment it is cut, each section should be laid in 1 per cent. osmic acid, in which it should remain from five to seven minutes; afterwards being washed in water, and transferred to an iodine solution composed of 0·2 per cent. iodine dissolved in 1·64 per cent. solution of potassium iodide. After remaining in this twenty to thirty minutes, the sections should be placed for at least half an hour (sometimes up to twenty-four hours) in a 25 per cent., or even stronger, solution of sulphuric acid; and finally into a 25 per cent. solution of sulphuric acid which has been saturated with iodine and has had added to it a drop of Meyer's pyoktanin solution.[1] After about five minutes in this the sections should be sufficiently stained for observation, and may be transferred for the purpose to glycerine. If the walls are sufficiently swollen by the sulphuric acid, and the staining of the plasmic threads has been successful, the selected sections lend themselves well to study. Alike the surface view of the epidermis (especially if this is examined inner side upwards), but above all the sections through the inner primary cortex, show exceedingly delicate, deeply blue-stained, plasmic threads in the swollen closing membranes of the pits, by which the protoplasts of neighbouring cells are connected together. These connecting plasmic threads we will call **plasmodesms.**[2] Fig. 163 shows their distribution in the wall of a cortical cell of *Viscum album*.—Should the mistletoe not be available, similar results may be attained by like treatment of the cortex of

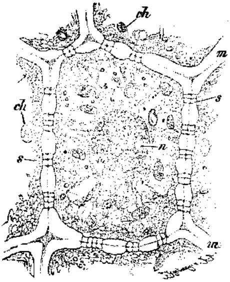

FIG. 163.—A cell from the cortex of Mistletoe (*Viscum album*) after suitable fixing and staining of the protoplasts and swelling of the walls (*m*). The closing membrane of the pit is traversed by plasmodesms; *ch*, chloroplasts; *n*, nucleus (× 1000).

[1] Meyer's pyoktanin solution is made with 1 gram of pyoktanin cœruleum, as sold by E. Merck, of Darmstadt, dissolved in 30 c.c. water. Pyoktanin is a very pure methyl violet.

[2] Plasmodesmen, plasmic bonds, a term introduced by Strasburger in 1901.—[ED.]

species of Fir (*Abies*).—According to Meyer only very thin sections are advantaged by swelling.—If precipitates form upon the surface of the sections, remove with a soft sable brush.

On account of the great ease with which it permits the plasmodesms to be brought to view, the endosperm of the vegetable ivory nut (*Phytelephas macrocarpa*) should also be studied ; these palm seeds are much used in manufacture, and can readily be procured. As the tissue of this endosperm is very hard, great care must be exercised in using the razor, and small sections should suffice. Examined under the microscope, these show a well-known structure, the same, in fact, as that of the stone of the date-palm referred to on page 73. If these sections are laid for a considerable time in dilute watery solution of safranin, or of pyoktanin, and then examined in glycerine, the stained plasmodesms can be easily distinguished within the closing membrane of the pits (Fig. 164). The plasmodesms traverse the closing membrane in a bowed form, so much the more strongly arched as they are farther removed from the centre. Besides these plasmodesms of the closing membrane, the endosperm of the date shows also others which traverse the entire thickness of the wall (*A*, *pl*). The identification of these is proportionately more difficult, and is made possible by suitable staining after the wall has been slightly swollen by the action of dilute sulphuric acid impregnated with iodine. In this way it will be seen that the peripheral cell-layers in the endosperm of *Phytelephas* show almost exclusively those plasmic connections which pass through the whole thickness of the wall; while the inner layers show also the pit connections as described. Where the section has so cut a wall that the pit is shown in surface, or full-face view, good staining of the sections may show the plasmodesms of the pit as a circular collection of coloured points or striæ (*C*).

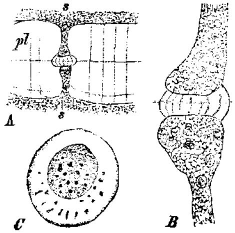

FIG. 164.—*A*, a slightly swollen piece of cell-wall from the endosperm of the vegetable ivory palm (*Phytelephas macrocarpa*). At *s* and *s* are the coincident pit-canals of two adjoining cells, filled with cytoplasm, and in the closing membrane are the delicate plasmodesms ; while other plasmodesms (*pl*) traverse the entire thickness of the cell-wall (× 375). *B*, contents of the two coincident pit-canals, and the plasmodesms of the closing membrane, without prior swelling (× 1500). *C*, full-face view of a pit-canal, and of the plasmodesm of the closing membrane (× 1500).

APPENDIX I.

ENGLISH AND METRIC WEIGHTS AND MEASURES.

THE following may be useful as an approximate transfer table.

LENGTH.

Metric.	*English.*
1 millimetre (mm.)	= $\frac{1}{25}$ inch.
1 centimetre (cm.) = 10 mm. . .	= $\frac{2}{5}$ inch.
1 decimetre (dm.) = 100 mm. . .	= 4 inches.
1 metre = 1,000 mm. . .	= $39\frac{1}{3}$ inches.
English.	***Metric.***
1 inch	= 25 mm.
1 foot	= 305 mm. or $30\frac{1}{2}$ cm.
1 yard	= 0·91 metre.

Microscopic measurements of size are usually reckoned in ***micro-millimetres***, represented by the Greek letter μ. 1 μ is $\frac{1}{1000}$ of a millimetre, and therefore is approximately $\frac{1}{25000}$ of an inch.

WEIGHT.

Metric.	*English.*
1 gramme	= $15\frac{1}{2}$ grains.
1 kilogram. = 1,000 gram. . . .	= 32 oz. Troy.
" " . . .	= $35\frac{1}{4}$ oz. Avoirdupois.
English.	***Metric.***
1 oz. Troy	= 31 gram.
1 oz. Avoirdupois	= 28 gram.
1 lb. "	= 450 gram.

CAPACITY AND WEIGHT.

Metric.	*English.*
1 gram. = 1 cubic centimetre (c.c.) .	= $15\frac{1}{2}$ grains.
1 litre = 1,000 gram. or 1,000 c.c. or 1 kilog.	= $35\frac{1}{4}$ oz. Avoird. or 32 oz. Troy.
English.	***Metric.***
1 pint = 20 oz. Avoirdupois . .	= $567\frac{1}{2}$ c.c. or $567\frac{1}{2}$ gram.

CAPACITY (VOLUME).

Metric.	*English.*
1 litre = 1000 c.c. = 1 cubic decimetre	= $1\frac{3}{5}$ pint.
English.	***Metric.***
1 pint = 36 cubic inches . .	= $567\frac{1}{2}$ c.c.
1 gallon = 8 pints . . .	= $4\frac{1}{2}$ litres.
1 cubic foot = 6 gallons . . .	= $28\frac{1}{3}$ litres.
1 cubic inch	= $18\frac{1}{3}$ c.c.

APPENDIX II.

LIST OF PLANTS AND MATERIALS USED FOR STUDY.

THE part of the plant required is carefully indicated in this list; likewise the state in which it should be taken, and the period at which it can be obtained. To these a few cultural notes are sometimes added.

Where the material is to be placed in alcohol, unless the word "absolute" is used, strong methylated spirit will serve, and is much cheaper. If possible this should be obtained of the "old style," which is freely miscible with water.

For fixing cell-contents, the quantity of alcohol, etc., used should be at least 100 times the bulk of the object.

Stems, etc., which are much hardened in alcohol, can be rendered more easy to cut by being placed for at least twenty-four hours in a mixture of half-and-half alcohol and glycerine.

Most of the Freshwater Algæ here mentioned can be obtained from T. Bolton, Newhall Street, Birmingham; and Marine Algæ from the Biological Station, Plymouth, or the Marine Biological Station, Port Erin, Isle of Man.

Messrs. Backhouse & Son,[1] the well-known nurserymen of York, have recently established on a large and complete scale a supply department for all kinds of botanical materials, living and preserved, under the direction of Dr. Arthur Burtt, and have undertaken to supply as far as possible all materials named in this work.

A.

Abies. *See Picea.*

Abutilon. Cork-formation in. Twigs, 182. Greenhouse shrubs.

Acacia. Compound pollen-grains, 391. Flowers. Fresh, or in alcohol. Greenhouse shrubs, mostly flowering in spring or early summer.

— Bastard, *see Robinia.*

Acer (Maple). Autumnal coloration of leaves, 58. Leaves in autumn. Fresh.

Aconitum Napellus (Monkshood). Structure of ovule, 398. Full-blown, or faded, flowers. Fresh. Summer. Hardy perennial.

(Other species of *Aconitum* will serve equally well. Most flower in June, July or August.)

[1] Now transferred to the "British Botanical Association, Limited," Holgate, York.

Acorus Calamus (Sweet Flag). Structure of root, 165. Roots. Fresh, or in alcohol. Hardy, semi-aquatic, readily grown at the edges of pools or slow streams.

Adonis æstivalis, red cell-sap in, 57. A hardy annual, flowering in June.

— *autumnalis*, 57. A hardy annual. Flowers in May.

Æcidium Berberidis (Cluster-cup), 330. Infected leaves of Barberry, in May or July. Fresh, dry, or in alcohol. *See also Puccinia graminis.*

Æsculus Hippocastanum (Horse-chestnut). For mechanism of fall of leaves, 201. Base of leaf-stalk with piece of twig attached. Early autumn. Fresh, or in alcohol.

— — Glandular hairs, 99. Winter buds. Preferably fresh; or in alcohol.

— — Pollen development, 444.

Agapanthus umbellatus. Development of anther and pollen, 385; of pollen, 393. Greenhouse half-hardy herbaceous perennial, flowering, indoors or out, from April to August.

Agaricus campestris (Mushroom). Structure, 238. Any time in the year. Fresh, or preferably in alcohol.

— — Reproduction, 336. Any time in the year. Fresh, or in alcohol.

Agave. Epidermis and stomata, 86. Leaves. Fresh. Any time. Greenhouse perennials. (Substitute for *Aloë*).

Ailanthus glandulosa. For induced leaf-fall, 203. Leaf-bearing twigs. Fresh.

Alder. *See Alnus glutinosa.*

Alisma Plantago (Water Plantain)[1] Structure of fruit, seed and embryo, 413. Ripe and unripe fruits. Fresh. July and August. Native semi-aquatic, by water-side.

Allium Cepa (Onion). Structure of root, 163. Roots. Fresh, or in alcohol. Obtainable at any time by growing an onion in water in a hyacinth glass. The pollen of the onion can be used for pollen-tube production, 392.

Alnus (Alder). Eight or ten year old twig. Fresh; used for estimating length and continuity of tracheæ, 161. A native tree.

Aloë nigricans. Epidermis and stomata, 86. Leaves. Fresh. Any time. Greenhouse perennial.

(Other species, or *Agave*, serve equally well).

Alstrœmeria. Cell and nuclear division, 439. Flower-buds of various ages. Fresh; also fixed and in alcohol (*see* p. 462). (*A. aurantiaca*, the commonest species, is a hardy perennial, flowering July to Sept.)

Althæa rosea (Hollybock). Pollen-grains, 388. Flowers. Fresh. July to September or October. Hardy perennial.

[1] *Alisma*, and most other water-side plants can be readily grown in an ordinary garden in a tub or old galvanised bath partially filled with soil, and kept supplied with water during the period of active life of the plants.

Ampelopsis hederacea (Virginian Creeper). Autumn tints, 58. Leaves Fresh. Autumn. Hardy climber.

Anabæna Azollæ. Structure, 261. Exists in the leaves of *Azolla carolineana.* Fresh. Any time. *Azolla* is a perennial greenhouse aquatic.

Anagallis (Pimpernel). Structure of ovary, 397. Flowers. Fresh, or in alcohol. Summer. *Anagallis arvensis* is a cornfield annual, with scarlet flowers; *Anagallis tenella* is a very pretty creeping native bog-plant.

Anaptychia ciliaris. A lichen found on tree stems. Structure, 239. Thallus. Fresh; or dried, but soaked in water before use.

— — Fructification, 336. Fruiting thallus. Fresh; or dried, but soaked in water before use.

Antirrhinum majus (Snapdragon). Coloured cell-sap, 56. Flowers. Fresh. May to September. Hardy perennials.

Apple. See Pyrus Malus.

Aquilegia. Summer flowering hardy herbaceous perennials. Used for pollen-cultures, 393.

Aristolochia Sipho. Structure of stem, 131. Young stems, $\frac{1}{8}$ to $\frac{1}{4}$ and $\frac{3}{8}$ inch thick. In alcohol; material to be put up in June or July. Hardy deciduous, or half-evergreen, climber. For substitute in young state, *see Ricinus.*

Arrowroot, East Indian, for starch, 18. Chemists.

Ascobolus furfuraceus. A discomycetous fungus, obtained upon horse or preferably cow dung, 327.

Ash. *See Fraxinus excelsior.*

Asparagus. Red chromatophores in the berry, 55. Late summer. Fresh. Gardens.

Aspidium Filix-mas (Male Fern). Fructification, 355. Fertile frond. Preferably fresh, but may be in alcohol. Summer. Native Fern.

Auricula. See Primula.

Avena sativa (Oat). Starch grains, 18. Grains. Dry.

— — Structure of vascular bundle, 113. Stems. In alcohol. Spring or early summer. Can be grown in laboratory for the purpose.

— — Puccinia on, 332. Leaves or haulm. Fresh, dry, or alcohol. Summer.

Azalea. Compound pollen-grains, 391. Flowers. Fresh. (For substitutes *see Calluna, Erica, Rhododendron.*)

— Vascular bundle ends, 199. Petals. Fresh, or in alcohol. *A. indica*, a spring flowering greenhouse shrub is best.

Azolla, for *Anabæna, q.v.*

B.

Bacillus buccalis, in the "fur" of teeth, 276.

— *subtilis*, 277. From infusion of hay. For method, *see* Text.

Bacteria, 266. To obtain materials, *see* Text.

Bacterium Termo. Obtained from the decay of peas and beans, 281.

Barberry. *See Berberis vulgaris.*

Barley. *See Hordeum vulgare.*

Bean. *See Phaseolus vulgaris.* Horse Bean; *see Vicia Faba.*

Beech. *See Fagus sylvatica.*

Beet-root. *See Beta vulgaris.*

Begonia. Crystals in, 64 (footnote). Stems; in alcohol.

Berberis vulgaris (Barberry). A hardy shrub, upon which *Æcidium Berberidis* (*q.v.*) may sometimes be found in spring or early summer, 330.

Beta vulgaris (Beet-root). Structure of root, 62. Root. Fresh. Used also for sugar in root, and for nitrates, 67.

Bracken-fern. *See Pteris aquilina.*

Brassica Napus (Rape), for development of flower, 429. A common weed of cultivated fields, flowering in summer.

Bryonia dioica (White Bryony). Substitute for *Cucurbita*, *q.v.* 126. A not uncommon hedge-row climber in some districts.

C.

Calluna vulgaris (Ling). Compound pollen-grains, 391. Flowers. Fresh, or in alcohol. July to September. A native sub-shrub.

Capsella Bursa-Pastoris (Shepherd's Purse). Structure and development of embryo and seed, 409. Young to ripe fruits. Fresh. All summer. Common weed of cultivated ground.

Carrot. *See Daucus Carota.*

Celandine. *See Chelidonium majus.*

Cerasus Lauro-Cerasus (Cherry Laurel). For pits in cortical cells, 73. Twigs. Fresh, or in alcohol.

Ceratopteris thalictroides. Cultivation of spores and development of prothallium, 362. Spores. Fresh, or preserved dry.

Chara fragilis (Stonewort). For reproduction, 306. Fresh. For structure, 246.

Cheiranthus Cheiri (Wallflower). Hairs, 91. Young leaves and buds. Fresh, or in alcohol.

Chelidonium majus (Celandine). Structure of vascular bundles; latex system, 124. Stems. In alcohol. Spring and summer. Hardy herbaceous perennial.

Cherry. Substitute for plum (*Prunus domestica, q.v.*).

— Laurel. *See Cerasus Lauro-Cerasus.*

Citrus vulgaris (Orange). Structure of fruit, 427. Fresh. All the year round.

Cladonia rangiferina (Rein-deer Moss). Substitute for *Anaptychia, q.v.*, 338.

Cladophora glomerata. Structure, 246. Fresh material; or fixed and preserved. Spring and summer.

— — Swarmspores, 293. For method of securing, *see* Text.

Club-moss. *See Lycopodium.*

Cluster-cup. *See Æcidium* or *Puccinia.*

Convallaria majalis (Lily of Valley). For pollen cultivation, 392. Fresh flowers.

Coprinus. For structure of hymenomycete, 336. Fresh, on dung-cultures.

Cowslip. *See Primula.*

Cratægus (Hawthorn). For orange-red chromatophores, 55. Fruits. Fresh.

Cucurbita Pepo (Cucumber, Melon, etc.). Movement of protoplasm in hairs, 47. Very young shoots. Fresh.

— — Pollen-grains, 390. Flowers. Fresh.

— — Structure of vascular bundles, and especially for sieve-tubes and sieve-plates, 126. Stems about $\frac{1}{3}$ inch thick, cut about $\frac{1}{2}$ yard from apex. Fresh, and in alcohol; or fixed with boiling water before cutting off.

Cytisus Laburnum (Laburnum). Structure of cork, 185. Fragments of bark from pretty old branches.

— — — For vascular bundle ends. Flowers, fresh or in alcohol, 199.

D.

Dahlia variabilis (Dahlia). Structure of tuber, 68. Tuber. Fresh. Any time.

— — Inuline, 69. Pieces of tuber placed in alcohol in or about October.

Dandelion (*Taraxacum Dens-leonis*). For latex system, 125. Roots, fresh or in alcohol.

Daucus Carota (Carrot). Colour-bodies in root, 57. Root. Fresh.

Delphinium Ajacis (Larkspur). Structure of ovary, 394. Fading flowers. Fresh. Summer. A hardy annual. (As substitutes, *see Helleborus niger* and *H. fœtidus.*)

— *consolida* (Larkspur). Coloured cell-sap and colour-crystals, 57, 58. Flowers. Fresh. Summer. Hardy annual. Also for pistil, 322.

Dracæna rubra (*Cordyline rubra*). Structure of stem, 117. Stems. Fresh, or in alcohol.

E.

Echeveria. Structure of chlorophyll-bodies, 53. Wax-layer, 99. Leaves. Fresh. All the year round. Almost any species will do. All half-hardy evergreen perennials, largely used in "bedding-out".

Elæagnus angustifolia (Oleaster, or Wild Olive). Hair-scales, 94. Leaves Fresh. (Substitute for *Shepherdia canadensis, q.v.*)

Elder. *See Sambucus nigra.*

Elodea canadensis. For protoplasmic movements, 49. Fresh.

— — For growing points, 212. Fresh.

Epilobium (Willow-herb). Substitute for *Œnothera biennis, q.v.*, 387, 397.

Equisetum arvense (Field horse-tail). Apical cell, 215. Young growing shoots. Fresh, or in alcohol. Spring.

— — Structure of stem, 179. Stems. Fresh, or alcohol-material.

Equisetum limosum. For reproduction, 363. Cones, fresh ; in ditches, June and July.

Erica (Heath). Compound pollen-grains, 391. Flowers. Fresh. Any species will do, and hence obtainable nearly all the year round, either from open ground or greenhouse. (For substitutes, *see Calluna, Azalea, Rhododendron*).

Eucalyptus globulus (Australian Blue-gum). Wax-layer, 99. Leaves. Fresh. Half-hardy perennial evergreen tree.

— — Substitute for *Ruta* (*q.v.*) for leaf structure, 195.

Euonymus japonicus (Spindle-tree). Growing apex, 212. Terminal buds. Fresh, or in alcohol. All the year round. Ornamental evergreen shrub ; half-hardy in colder districts.

Euphorbia helioscopia (Sun-spurge). Starch-grains, 19. Stems. Fresh. A native annual weed of cultivated ground.

— — For crystals of calcium phosphate. Inflorescence in alcohol, 70. Summer. Also found in the greenhouse *E. Caput-Medusæ*, in ground tissue of stems.

— *splendens* and *E. Jacquinæflora.* Starch-grains and latex, 19 ; latex-cells. Stems. Fresh, or in alcohol. Hothouse evergreen shrubs.

F.

Fagus sylvatica (Beech). Differing structure of leaves when shaded and exposed, 195. Leaves from centre and outside of tree. Fresh, or in alcohol.

Ferns. Prothallia and sexual organs. *See Ceratopteris* and *Polypodium.*

— Sporangia. *See Scolopendrium, Aspidium.*

— Structure of growing apex of root. *See Pteris cretica.*

— Structure of stele. *See Pteris aquilina.*

Ficus elastica (India-rubber tree). For cystoliths, 64 (footnote). Leaves. Fresh. A popular greenhouse decorative plant.

Fir, Scotch. *See Pinus sylvestris.*

Flag, Sweet. *See Acorus Calamus.*

Fraxinus excelsior (Ash). Leaf-fall, 203. Leafy twigs. Fresh. Can be used as substitute for *Æsculus.* For growing points, as substitute for *Euonymus*, 215.

Fritillaria imperialis (Crown Imperial). Structure. Pollen, 386. Young flowers. Alcohol-material.

— *persica.* Cell and nuclear division, 439. Flower-buds of different ages. Fresh, and fixed, 462. (As substitute, almost any species of *Fritillaria, Lilium, Alstrœmeria*, or other Liliaceæ or Amaryllideæ.)

Frog-bit. See *Hydrocharis.*

Fuchsia. For water-pores, 89. Leaves. Pollen-grains, 387. Flowers. Fresh. (Substitute for *Œnothera, q.v.*)

Fucus platycarpus and *F. vesiculosus.* For structure, 240. Fresh, or in alcohol.

Fucus platycarpus and *F. vesiculosus.* For reproduction, 298. Preferably fresh.

Funaria hygrometrica. Chlorophyll-bodies, 52. Leafy stems. Fresh. All the year round.

— — Sexual organs, 347. Male and female plants. Fresh, or in alcohol. Plants with the sexual organs and the sporogonia in all stages of development can be found nearly all the year round.

Funkia ovata. Development of pollen, 385. Flower-buds of different ages. May. Fresh, or in alcohol. (Substitute for *Hemerocallis, q.v.*)

G.

Gall, Oak. Structure and tannin reaction, 70. Fresh, or dried.

Ginkgo biloba. For autumn tints. Fresh leaves, 58.

Glœocapsa caldariorum, 263 (or *G. polydermatica*). Fresh. On walls, flower-pots, glass, etc., in greenhouses and fern-houses. All the year.

Gloxinia hybrida. Pollen-cultures, 392. Embryo-sac, 405. Flowers. Fresh.

Gymnocladus canadensis. Leaf-fall, 203. Leafy twigs. A very ornamental hardy deciduous tree. Prefers shaded position. (Used as substitute for *Æsculus, q.v.*)

Gymnogramme. For culture of fern-spores, 363.

H.

Hart's-tongue fern. *See Scolopendrium.*

Hedera Helix (Ivy). Resin canals, 152. Young twigs. Fresh, or in alcohol.

Helianthus annuus (Sunflower), and *H. tuberosus* (Jerusalem artichoke). For vascular bundles. (Substitute for *Aristolochia, q.v.*)

Hellebore. *See Helleborus.*

Helleborus fœtidus (Stinking Hellebore). Cell and nuclear division and pollen-formation, 443. Flower-buds of various ages. Fresh, or in alcohol.

— — Structure of ovary, 395. Flowers. February and March.

— *niger* (Christmas Rose). Structure of ovary, 395. Flowers. January, February.

(These two substitutes for ovary of *Delphinium, q.v.*)

Hemerocallis fulva. Development of anther and pollen, 380. Flower-buds of different ages. Fresh, and in alcohol. Summer. A hardy herbaceous perennial. (As substitutes, *Lilium, Funkia, Agapanthus umbellatus, Tulipa, Hyacinthus.*) Also for pistil, 396.

Hibiscus. For cork, 182. Stems. Greenhouse perennial.

Hippuris vulgaris (Mare's-tail). Structure of growing apex, 204. Growing buds. Fresh, or in alcohol. Late spring or early summer. A native perennial herbaceous marsh or aquatic plant.

Hollyhock. *See Althæa rosea.*

Hordeum vulgare (Barley). Structure of growing apex of root, 220. Roots of plants grown in flower-pots.

Horse-chestnut. *See Æsculus.*

Hyacinth. *See Hyacinthus.*

Hyacinthus. Structure of ovary, 396. Full-open flowers. Fresh. (As substitutes, *see Tulipa, Lilium,* or other Liliaceæ.)

— Development of pollen, 385. (Substitute for *Hemerocallis, q.v.*)

— Also used for structure of stomata, 84; and for stele of root, 165.

Hydrocharis Morsus-Ranæ (Frog-bit). Movement of protoplasm in root-hairs, 47. Young roots. Quite fresh. A native aquatic, with floating rosettes of heart-shaped leaves; easily grown in still water.

I.

Impatiens parviflora. For vascular bundle ends, 200. Leaves, in alcohol.

Indian corn. *See Zea Mais.*

India-rubber plant. *See Ficus elastica.*

Iris florentina. Structure of leaf, 80. Leaves. Fresh, and in alcohol.

— — Wax, 80, 99. Leaves. Fresh.

— — Endodermis of root, 166. Roots. In alcohol.

— — Vascular bundle, 114. Leaves. In alcohol.

— *germanica.* Starch-builders (leucoplasts) and starch, 59. Surface rhizomes. Fresh. Both species are hardy, herbaceous, more or less evergreen.

— — Any species, used as substitute for *Hemerocallis* in development of pollen, 385.

Ivy. *See Hedera Helix.*

J.

Juglans regia (Walnut). Leaf-fall, 203. Leafy twigs. Fresh. *See* also as in *Æsculus.*

L.

Laburnum. *See Cytisus Laburnum.*

Larkspur. *See Delphinium.*

Lathyrus (Sweet Pea, Everlasting Pea). Formation of pollen-tube, 392. Freshly opened flowers.

Lemna (Duckweed). For general structure of root, 219. Fresh.

Lepidium sativum (Cress). Used as host-plant for *Pythium*, 321. Seedlings grown crowded together and kept very wet.

Leptothrix buccalis, 276. In the "fur" on teeth.

Leucojum (Snowflake). Development of pollen-grains of, 386. Flower-buds of different ages. Fresh, and in alcohol. (As substitute for *Tradescantia, q.v.*) Also for development of pollen-tubes, 392.

Lilium (Lily). Used as *Leucojum* above, 385.

— Structure of ovary, 396. Fully developed flowers. Fresh. Structure of ovule, 405.

Lilium. Cell and nuclear division, 439, 462. Flower-buds of different ages. Fresh, and in absolute alcohol. (As substitutes, *Fritillaria*, *Alstrœmeria*.

— *candidum*. For stomata, 86. Leaves. Fresh, or in alcohol.

Lime. *See Tilia*.

Liverworts. *See Marchantia*.

Lupinus albus (Lupine). Aleurone grains, 32, 34. Seeds. Dry.

Lycopersicum esculentum (Tomato). For red chromatophores, 55. Fruit. Fresh.

Lycopodium complanatum (Club-moss). Structure of stem, 176. Stems. Fresh, and in alcohol.

Lysimachia (Loosestrife). Development of pollen-tubes, 393. Structure of ovary, 397. Fully developed flowers. Fresh. (Substitute for *Primula*.)

M.

Maize. *See Zea Mais*.

Malva crispa. Pollen-grains, 388. Flowers. Fresh. (Substitute for *Althœa rosea*, Hollyhock, *q.v.*)

Maple (*Acer*). Autumn coloration, 58. Leaves. Fresh.

Marchantia polymorpha. Vegetative structure, 233. Thallus. Preferably fresh, or in alcohol.

— — Reproductive organs and sporogones, 339. Receptacles. Fresh, and in alcohol. June to August.

Mare's-tail. *See Hippuris vulgaris*.

Matthiola annua (Ten-week Stock). Hairs, 92. Leaves. Fresh, and in alcohol. Late spring and summer.

Mnium hornum. Reproductive organs and sporogonia, 345. May and June. Fresh, or in alcohol.

— *undulatum*. Vegetative structure, 228. Fresh. (As substitutes, *Mnium hornum*, or *Polytrichum*.)

Monkshood. *See Aconitum Napellus*.

Monotropa Hypopitys (Bird Rape). Structure of embryo-sac, 400. Flowers. Fresh. Found occasionally in woods, etc., especially under beech trees; flowers in July and August. It should be examined fresh, as it becomes brown and opaque in alcohol. It bears transport very well, and can be preserved fresh for some time in a glass of water.

Morchella esculenta (Morell). Vegetative structure and cell-contents, 326. Fresh or dry.

Morell. *See Morchella esculenta*.

Mosses. *See Mnium, Polytrichum, Funaria*.

Mucor Mucedo (Pin-mould). Structure and reproduction, 311. Found in a few days on a piece of damp bread placed under a bell-glass, or on fresh horse-dung similarly placed. For zygote production, see p. 315.

Mullein. *See Verbascum*.

Mushroom. *See Agaricus campestris.*

Myriophyllum. For growing points, 212. Substitute for *Hippuris* (*q.v.*).

N.

Narcissus poeticus. For structure of ovule and ovary, 405. Flowers. Fresh, or in alcohol.

Nasturtium. *See Tropæolum.*

Nettle, stinging. *See Urtica.*

Navicula. *See Pinnularia.*

Nitella. Rotation of protoplasm in, 50; vegetative structure, 245. Fresh plants. *Nitella* can be grown in glass vessels of water, especially if fed with the culture-fluid given on page 251.

— Reproduction, 309. Plants. Fresh.

Nostoc ciniflonum, 262. Fresh. Sometimes found in large olive-green masses on damp paths. In some parts of the country known as "witches' butter".

O.

Oak. *See Quercus.*

Oak-gall. *See* Gall.

Oat. *See Avena sativa.*

Œnothera biennis (Evening Primrose). Pollen-grains and development of pollen-tube, 386. Flowers. Fresh. Summer. (As substitutes, *Epilobium*, *Fuchsia*, *q.v.*)

— — Structure of adherent ovary, 397. Fresh, or in alcohol.

Onion. *See Allium Cepa.*

Orange. *See Citrus vulgaris (C. Aurantium).*

Orchideæ, Ovary, 392. *See Epipactis.*

Embryo-sac, 404. Flowers some time faded. Fresh. (Substitute for *Monotropa*, *q.v.*)

Ornithogalum unbellatum (Star of Bethlehem). Structure of cell-walls of seed, 72. Seeds. Dry.

Oscillaria, 262. Stagnant water, muddy ground, etc.

Osmunda regalis (Royal Fern). For germination of spores and formation of prothallia, 362.

P.

Pæonia (Pæony). Formation of pollen-tubes, 392. Flowers. Fresh. Pollen-grains grown in 5 per cent. sol. of sugar, and 1·5 per cent. gelatine.

Pansy. *See Viola tricolor.*

Papaver Rhœas (Field Poppy). Structure of petals, 199. Petals. Fresh, or in alcohol.

Parmelia ciliaris. *See Anaptychia.*

Pea. *See Pisum sativum.*

Pear. *See Pyrus communis.*

Pelargonium zonale (so called "Geranium"), for glandular hairs, 97. Petioles. Fresh, or in alcohol.

Pellionia Daveauana. Green starch-formers in young stem, 60. Fresh.

Penicillium crustaceum (Blue Mould), 323. Obtained on a piece of moist bread under a bell-jar.

Peronosporeæ. *See Phytophthora.*

Phajus. Half-grown pseudo-bulbs used for leucoplasts, 59. Fresh, or fixed with picric acid.

Phaseolus vulgaris (Bean). Starch, 17. Bean flour; or obtained by scraping a cut bean.

Phœnix dactylifera (Date). Structure of endosperm, 73. Date-stones.

Phycomycotes. *See Mucor Mucedo.*

Phytelephas macrocarpa (Vegetable Ivory). Seeds, for protoplasmic interconnections, 466. Fresh.

Phytophthora infestans (Potato disease), 318. Diseased leaves of potato. Fresh.

Picea vulgaris (*P. excelsa*). Female cones and fertilisation, 372. Cones. Alcohol. Mid-June. Fertilisation is completed in June; the exact date for the locality varies from year to year. Hence cones should be gathered daily from 1st June, and the scales, separated from one another, placed in absolute alcohol. Before investigation the scales must be laid for at least twenty-four hours in a mixture of equal parts of glycerine and water.

Pilobolus crystallinus. On dung cultures, 327.

Pinnularia viridis, 255. Fresh. Not infrequent in standing and running water.

Pinus sylvestris. Bordered pits, 74 *et seq.* Pieces of old stem in alcohol.

— — Structure of stem and development of bordered pits, 137. Young stems, and pieces of outer part of old stems, cut in June or July, and laid in alcohol. To be placed in glycerine and alcohol before using.

— — Male flowers, 367. Male cones. Alcohol. May or early in June. Laid in glycerine and alcohol before using.

— — Female flower, ovule, 370. Young cones. Alcohol. May or June. Glycerine and alcohol before using.

— — Pollination, 371. Young cones as above, but fresh.

Pisum sativum (Pea). Structure of seed, and aleurone grains, 25. Ripe peas. Dry.

Pleurosigma angulatum, 259. A diatom used widely as a test-object.

Plum. *See Prunus domestica.*

Polypodium vulgare (Polypody fern). Reproduction, sexual and asexual, 357. Prothallia and fertile leaves. Fresh.

Polytrichum juniperinum. Structure of stem, 230. Stems. Fresh, or in alcohol.

— — Antheridia, 346. Plants in "flower". May. Fresh, or in alcohol.

Potato. *See Solanum tuberosum.*

— starch. Commercial, from a chemist.

Primrose. *See Primula.*

Primula. Ovary, 397. Flowers of any species.

— *sinensis.* Glandular hairs, 97. Leaf-stalks. Fresh.

Protococcus viridis, 259. On damp bark or walls. Fresh.

Prunus domestica (Plum). Structure of fruit, 423. Fruit. Fresh.

Pteris aquilina (Bracken-fern). Structure of stele, 172. Young leaf-stalks or slender rhizomes. Fresh, or in alcohol.

— *cretica.* Structure of root-apex, 224. Roots. Fresh, or in alcohol. Very commonly cultivated in pots; and roots can be best obtained unbroken by turning the plant out of the pot containing it.

Puccinia graminis (Rust-fungus), 330. Dry, or in alcohol. Found in summer on different kinds of cereals, and on *Triticum repens* (couch-grass).

Pyrola (Winter-green). Embryo-sac, 401. Flowers. Fresh. Herbaceous perennials, various species of which can be easily cultivated on a shady border, in sandy peat.

Pyrus communis. Stone-cells in fruit, 65. Fresh fruit.

— *Malus* (Apple). Structure of fruit, 425. Fresh fruit.

Pythium de Baryanum. A parasitic and saprophytic fungus causing the disease known as "damping off". Obtained upon cress seedlings (*q.v.*), 321.

Q.

Quercus pedunculata (Oak). *See* Gall.

— *Suber* (Cork oak). Structure of cork, 187. Bottle cork.

R.

Ranunculus repens (Creeping Buttercup). Structure of roots, 168. Roots. In alcohol.

— — Structure of vascular bundle, 121. Runners. In alcohol.

Rhododendron. Pollen, 391. Flowers. Fresh. (For substitutes, *see Azalea, Erica, Calluna.*)

Ribes rubrum (Red Currant). Structure and development of cork, 187. Young and older stems. Fresh, or in alcohol. Best gathered in July.

Ricinus communis (Castor Oil). Aleurone grains, 33. Seeds.

— — Stems of seedlings used for vascular bundles, as a substitute for *Aristolochia* (*q.v.*), 136.

Robinia Pseud-Acacia. Leaf-fall, 208. Treat as in *Æsculus.*

— — Old stems used for tyloses (tracheal plugs), 162. Fresh, or in alcohol.

Rosa semperflorens. Structure of prickles, 94. Young stems. Fresh. Also leaves. Fresh. Most sp. of *Rosa* will do.

Rose. Coloured cell-sap, 55. Petals. Fresh.

Rue. *See Ruta graveolens.*

Russula rubra, 333. Fresh, or in alcohol. (As substitute, *see Agaricus campestris.*)

Rust-fungus. *See Puccinia graminis.*

Ruta graveolens (Common Rue). Structure of leaf, 189 *et seq.* Leaves. Fresh. Obtainable all the year round. A hardy sub-evergreen.

S.

Saccharomyces Cerevisiæ (Yeast), 288. Can be grown in Pasteur's fluid.

Saccharum officinarum (Sugar-cane). For wax, 99. Stem. Frequently grown in hot-houses.

Salisburia adiantifolia. See Ginkgo biloba. A hardy deciduous tree.

Sambucus nigra (Elder). Cork, 182. Twigs of various ages. Fresh, and in alcohol.

Saxifraga crustata. For deposit of carbonate of lime on leaf, 64 (footnote). Fresh leaves. Common garden species of rock plant.

Scolopendrium vulgare (Hart's-tongue Fern). Structure of leaf, and sporangia, 354. Fertile leaves. Alcohol.

Scorzonera hispanica (Salsify). Latex system, 125. Roots. Fresh, and in alcohol. A hardy kitchen-garden herbaceous plant.

Scotch Fir. *See Pinus sylvestris.*

Selaginella Martensii. Structure and spore-production, 365. Fertile shoots. Dry, or in alcohol. This, or some similar species is universally cultivated in plant-houses.

Shepherdia canadensis. Scale-hairs, 93. Leaves. Fresh, or in alcohol. Hardy deciduous shrub.

Shepherd's Purse. *See Capsella Bursa-pastoris.*

Siphoneæ. See Vaucheria.

Solanum tuberosum (Potato). For structure of Starch, 10. Used for production of pathogenic cork, 187-8. For cultivation of bacteria, 266. In each case use fresh tubers. *See* also *Phytophthora.*

Sphagnum acutifolium (Bog-moss). Structure, 231. Plants. Fresh. Very commonly used in plant-houses.

Spindle-tree. *See Euonymus.*

Spirochæte plicatilis, 276. Water containing decaying algæ, especially *Spirogyra* and *Vaucheria.*

Spirogyra. Structure, 252. Living plants.

— Conjugation, 291. Living plants. Summer. Plants in this state are recognisable by the crinkled yellowish look, and clinging together of the masses of threads. For the culture of *Spirogyra, see* p. 251.

Staphylea. Formation of pollen-tube, 392. Flowers. Fresh. Grown in 5 per cent. solution of sugar, and 1·5 per cent. gelatine.

Stinging Nettle. *See Urtica dioica.*

Stock, Ten-week. *See Matthiola annua.*

Strelitzia regina. For orange-red chromatophores, 55. Flowers. Fresh. A showy-flowered warm-house perennial.

Sugar-cane. *See Saccharum officinarum.*

Sweet Pea. *See Lathyrus.*

T.

Tanacetum vulgare (Tansy). Pollen development, 444. Fixed.

Taraxacum (Dandelion). For anastomosing latex system, 125. Roots. Fresh, or in alcohol.

Taxus baccata. (Yew). Structure of root, 168; of root-apex, 222. Roots. Fresh, or in alcohol.

Thuja occidentalis. Growing apex of root, 222. Young roots. Best in alcohol.

Tilia parvifolia (Lime-tree). Structure of stem, 153. Branches and twigs. Fresh, and in alcohol. The latter best gathered in July.

Toadstool. *See Amanita.*

Torenia asiatica. Development of pollen-tubes, 392. Fertilisation, 405. Flowers. To study fertilisation, the flowers selected should be pollinised by hand a day and a half or two days before they are required. *Torenia* is a hot-house plant, treated as an annual, and flowering in June and July.

Tradescantia virginica. Movements of protoplasm, 38, etc. Flowers. Fresh. *T. virginica* is a hardy herbaceous perennial, flowering from May or June to September or October.

— — Stomata, 84. Leaves. (*T. zebrina*, a common plant in plant-houses can replace this.)

— — Structure of pollen-grains, 385. Flowers and buds of different ages. Fresh.

— — Development of pollen-tube, 392. Freshly opened flowers. Grown in a solution of 5 per cent. sugar and 1·5 per cent. gelatine.

— — Cell and nuclear division, 434. Fresh flower-buds between $\frac{1}{8}$ and $\frac{1}{4}$ inch high. Stamens should be examined in 3 per cent. sugar.

— — Direct nuclear division, 456. Old stems. Fresh.

— *zebrina.* Stomata, 85. Leaves. Fresh.

Trianea bogotensis. For circulation in root-hairs, 48. A warm-house aquatic.

Triticum durum (Wheat). Starch, 18. Wheat grains. Dry.

— *vulgare* (Wheat). Structure of grain; aleurone, 29; embryo, 417. Wheat grains.

Tropaeolum majus ("Nasturtium," or Indian Cress). Colour bodies, 54. Flowers. Fresh.

— — Water-pores, 88. Leaves. Fresh, and in alcohol.

Tulip. *See Tulipa.*

Tulipa. Development of pollen, 385. (Substitute for *Hemerocallis*, *q.v.*) Flowers. April and May. Fresh, and in alcohol. Development of pollen-tubes, 392.

— Structure of ovary, 396. Old flowers. Fresh, or in alcohol.

U.

Urtica dioica (Stinging Nettle). Stinging hairs, etc., 95. Young leafy stems. Fresh.

V.

Vallisneria spiralis. Movements of protoplasm, 48. Strong, rather old, leaves. Fresh. Very commonly and easily grown in aquaria.

Vaucheria sessilis. Structure and reproduction, 295. Strong plants taken from still or flowing water, and placed the day before wanted in a shallow vessel with fresh water.

Vegetable Ivory. *See Phytelephas macrocarpa.*

Verbascum nigrum (Mullein). Coloured cell-sap, 55, 92. Flowers. Fresh.

— — Hairs, 92. Flowers. Fresh, or in alcohol.

— — Ends of vascular bundles, 199. Flowers. Fresh, or in alcohol. A native herbaceous perennial, flowering in July and August.

— *thapsiforme.* Felted hairs, 93. Leaves. Fresh, or in alcohol.

Veronica serpyllifolia. For pollen-tubes *in situ*, 408. Ovaries of just-fallen flowers. Fresh.

Vicia Faba (Horse Bean). Germinated 2-4 days, 459. Fresh.

Vinca major, or *V. minor* (Periwinkle). Coloured cell-sap, 56. Flowers. Fresh.

— — Sclerenchyma fibres, 72. Stems. Fresh, or in alcohol. Native or introduced, perennial evergreen plants, flowering July to September.

Viola tricolor (Pansy). Hairs, 92. Flowers. Fresh, or in alcohol. Can be had from May to September.

— — Stipular glands, 98. Young stipules. Fresh, or in alcohol.

Virginia Creeper. *See Ampelopsis hederacea.*

Viscum album (Mistletoe). Intercellular protoplasmic threads, 464. Stems, fresh.

W.

Wallflower. *See Cheiranthus Cheiri.*

Wheat. *See Triticum.*

Y.

Yew. *See Taxus baccata.*

Yucca. Structure of ovary, 396. Ovaries. In alcohol.

Z.

Zea Mais (Maize). Structure of vascular bundles, 101. Young stems. In alcohol.

APPENDIX III.

REAGENTS AND MOUNTING MEDIA USED IN THIS WORK, AND HOW TO PREPARE AND USE THEM.

ALL the reagents and mounting media in this list can be obtained, ready made, of Messrs. Southall Bros. & Barclay, Manufacturing Chemists, Birmingham, or of Dr. George Grübler, Leipzig, Dufour-Strasse, No. 17. Or the materials can be obtained from the same sources, and the student will find in this Appendix instructions how to prepare them for use. It is hoped that the instructions are sufficiently explicit, hut the editor will be glad to have errors or inefficient descriptions pointed out to him.

Percentage solutions are made either by weight or volume; or, if the metric system (see Appendix I.) is used, by, within certain limits, either indiscriminately. Thus, 1 per cent. acetic acid in water is made by taking 1 volume acetic acid and adding to 99 volumes distilled water; 5 per cent. potash solution, by taking 5 grammes potash and adding to 95 c.c. distilled water (1 gramme weight of water = 1 cubic centimetre volume).

A saturated solution can be secured by seeing that some of the salt, etc., is always lying undissolved at the bottom.

Further information as to the uses of the reagents will be found by reference to the general index.

N.B.—For all purposes where alcohol up to 90 per cent. is used (*i.e.*, "alcohol," not "absolute alcohol,") strong methylated alcohol will serve. Its alcoholic strength varies from about 85 to 90 per cent. Percentage compositions can therefore be made with it instead of absolute alcohol.

Approximately 50 per cent. alcohol = 56 parts strong meth. alc., 44 water.
60 per cent. alcohol = 67 parts meth. alc., 33 water.
70 per cent. alcohol = 78 parts meth. alc., 22 water.
82 per cent. alcohol = 91 parts meth. alc., 9 water.
(As this is a rather special strength it is best prepared with absolute alcohol.)
90 per cent. alcohol = strong methylated alcohol.

If possible "old style" methylated spirit should be obtained, as it alone mixes freely with water.

The following table of alcoholic strengths may be of utility:—

Sp. Gr. 0·830	= 61 over proof	= 91·3 per cent.		
" 0·820	= 65 "	" = 94·0	"	
" 0·810	= 68 "	" = 96·0	"	
" 0·805	= 71 "	" = 97·5	"	
" 0·794	= 75 "	" = 100·0	"	(Absolute).

A.

Acetic acid, Glacial. Used as reagent for fat oils, 34.

— — 1 *per cent.* For fixing the nucleus, especially in combination with methyl-green or gentiana-violet (*q.v.*), and as an addition to the mountant in mounting carmine preps., 250.

— — 2 *per cent.* For fixing and defining nuclei, 404.

Acetic acid is an excellent clearing reagent for rendering tissues transparent, and showing up the cell-walls, 210, 216, 270. It is likewise used to distinguish crystals of oxalate of lime as they are not soluble in it, 63. In very dilute solution (0·5 per cent.) it is useful for somewhat decolorising overstained preps., 272, 274.

— — *and Gentiana-violet. See* Gentiana-violet.

— — *and Methyl-green. See* Aniline-green, Acetic.

Agar-Agar. Obtained from *Eucheuma gelatinæ*, or *Gigartina speciosa*, is used in the East in the place of ordinary gelatine for preparing soups and jellies. It bears, without liquefying, higher temperatures than ordinary gelatine. It is a culture material for bacteria, 283; and in ½ per cent. solution brings on conjugation in *Spirogyra*, 292.

Alcohol, Absolute. Where the alcohol should contain a very specific proportion of water, it is better to use absolute alcohol diluted, as ordinary spirit varies slightly in strength, and almost always contains acids. Thus, material preserved for working with carbonate of lime crystals (cystoliths) always shows these dissolved if kept in ordinary spirit. Where expense is an object, methylated spirit—only about one-tenth the price—can however for most purposes be used.

— 50 *per cent.* Used with alkanet for resin reaction, etc. *See* 144; also for making picric alcohol, and picric aniline-blue, and magenta solution (*q.v.*).

— 82 *per cent.* Used to harden celloidin in. *See* 400.

— *Acidulated.* 70 per cent. alcohol + 0·5 per cent. hydrochloric acid. Used in overstaining. *See* 273.

— *and Glycerine*, half and half. Used for softening hard tissues preserved in alcohol, and thus preparing them for sectionising. From twenty-four to forty-eight hours suffices.

— *Methylated. See* above under Absolute Alcohol.

— *Picric.* Picric acid dissolved in 50 per cent. alcohol. An exceedingly good fixing and staining reagent for filamentous algæ. Needs careful washing out if staining with logwood is desired. *See* p. 248.

Alkanet root and alcanna tincture. Used for resin reactions. The alcoholic tincture of alcanna is added to so much water that the resin will not dissolve in it, or a thin chip of Alkanet root, washed in water to remove dust, can be placed with the preparation, and 50 per cent. alcohol run under the cover-glass. The resin drops may take so long as an hour to colour deep red, 144. The coloured drops are

dissolved in strong alcohol. Alcanna-tincture also stains protoplasm pale rose red, from a quarter to half an hour being needed. It is sometimes used to identify the ground substance of oil-containing seeds. Fats colour deep red; cuticle and cork both slowly colour red, in varying degrees, in solution of alkanet in 50 per cent. alcohol.

Alum, watery solution. Restores a section which has been "cleared" too completely, 210. Also in 1 per cent. solution to restore overstaining, 249.

— 10 *per cent.* Used as a mordant, 111.

Alum-carmine, Grenacher's. *See* Carmine.

Ammonia, strong watery solution. Used often instead of potash for clearing tissues, etc. Also, after the use of nitric acid, to produce the yellow colour in protoplasm, known as the Xanthoproteid reaction. By the same reaction the middle lamella of thick-walled tissue is stained yellow. Ammonia is also of great service in softening dry herbarium material, preparatory to microscopical examination; and to neutralise acids. *See* also p. 161.

Ammonia hæmatin. For method of preparation and use, *see* p. 249. Used for staining the cell-contents of filamentous algæ which have been fixed in picric acid. A rather troublesome method, but with often very beautiful results.

Aniline-blue, dilute watery solution, acting for up to half an hour. Used as a reagent for staining the callus of sieve-plates in sieve tubes, which it stains more or less permanently. Glycerine dissolves it out from ordinary cell-walls. It also stains protoplasm, often deeply. Eosin is an excellent subsequent stain.

— (*Hoffmann's Blue*), dissolved in 50 per cent. alcohol and containing 1 per cent. acetic acid, can be used for the same purpose. It likewise stains protoplasm and not the cell-wall, as the colour can be removed from the latter by washing in water and mounting in glycerine. Alcohol material must be washed with water before staining. *See* also Methyl-blue (or Methylene-blue)

— *with picric acid.* *See* Picric aniline-blue.

Aniline-green (Acetic). Dissolve aniline-green in 1-2 per cent. solution of glacial acetic acid in distilled water until the solution is of a clear blue-green colour. Especially good for fixing and staining the nucleus in its stages of division. *See* p. 438 *et seq.* The colour is not permanent.

— *oil.* Used in preparing Ehrlich's aniline water fuchsin, 273.

— *sulphate or chloride.* Test for lignin. In dilute watery, or in alcoholic solution, alcoholic chloride being best. Treat the section first with this, and lignified walls are stained yellow. If the section is subsequently treated with dilute sulphuric or dilute hydrochloric acid, the colour is deepened. Or a mixture of the solution with $\frac{1}{10}$ its bulk of sulphuric or hydrochloric acid can be kept.

— *water fuchsin* (Ehrlich's). *See* p. 273 for preparation and use.

B.

Bismarck brown, watery solution. Especially used for staining Bacteria, 270.

Borax-carmine (Grenacher's). *See* Carmine.

Bromine vapour. Sometimes used in fixing marine algæ, 302.

C.

Camphor. For use as a slight antiseptic. *See* pp. 60 and 374.

Canada balsam. Dissolved to the thickness of syrup, in turpentine, chloroform, benzole, or xylol. It can be obtained dissolved in turpentine in metal tubes like those used for liquid colours, and is very convenient in this form. Used as a mountant, but not so generally useful as glycerine jelly (*q.v.*). When used the section should be thoroughly dehydrated before mounting, hence stains which are soluble in alcohol are rarely of the least use with it.

Carbolic acid. In alcoholic and watery solutions, used for clearing preparations, and is often better than potash.

— *fuchsin (Ziehl's)*. For preparation and use, *see* p. 273.

Carmine. Solutions of carmine usually colour diffusely; but the nuclei usually show very well stained if the preparations are afterwards treated for some time with acidulated alcohol (*q.v.*), or with acidulated glycerine, *i.e.*, glycerine containing ½ per cent. hydrochloric acid. Pure carminic acid can be obtained of E. Merk, in Darmstadt.

Carmalum (Mayer's) is perhaps the best carmine stain, and is prepared by dissolving by the aid of heat 1 gramme pure carminic acid and 10 grammes alum in 200 c.c. distilled water; filter, and add ·1 per cent salicylic acid as a preservative. To get a pure nuclear stain wash the preparation carefully with a solution of alum or weak acid.

Carmine, Alum (Grenacher's). A 1-5 per cent. watery solution of common or ammonia-alum is boiled for from ten to twenty minutes with ½ to 1 per cent. powdered carmine, and, after cooling, is filtered. A trace of carbolic acid is added. (*Archiv. f. mikr. Anat.*, xvi., p. 465.)

— *Borax (Grenacher's)*. 2-3 per cent. carmine dissolved by boiling in solution of 4 per cent. borax in water; dilute with an equal volume of 70 per cent. alcohol, shake, and filter after allowing it to stand for some time. After using this stain it is recommended to treat for about twenty-four hours in a 2-4 per cent. solution of oxalic acid in 70-80 per cent. alcohol. It is an excellent stain for protein cell-contents, but is slow—sometimes requiring several hours. It can be used for staining in mass as well as for sections.

Cedar, Oil of. Used for clearing preparations of bacteria, prior to mounting in Canada balsam or dammar. *See* pp. 271 and 449.

Celloidin. A form of collodion. For use, *see* p. 400.

Cherry-wood extract. Twigs of cherry, rejecting the thin, green parts, are cut up into thin shavings, steeped in absolute alcohol for twenty-four hours, to remove the chlorophyll as much as possible. The

shavings are now shaken free of the discoloured alcohol and steeped in a new supply, and allowed to remain for several days, being frequently stirred. This fluid is then filtered, and evaporated down until a fragment of very coarse unbleached blotting-paper moistened with it, and subsequently with hydrochloric acid, quickly becomes a deep violet colour. The residual fluid thus obtained is brown, and smells like camphor. Preserve in a well-closed bottle. Reagent for lignin, 79.

Chloral-hydrate. Used as a clearing reagent, of which it is one of the best, especially for growing points and pollen-grains. A solution of 8 parts in 5 parts water.

Chloroform. Solvent of fat and etherial oils.

Chlorophyll solution, freshly prepared and concentrated; a reagent for cork and cuticle, 185.

Chlorzinc iodine (Iodized chloride of zinc). Zinc is dissolved in pure hydrochloric acid, and the solution evaporated (metallic zinc being kept in it during the process) to the consistence of strong sulphuric acid; in this is dissolved as much iodide of potassium as it will take up, and finally as much metallic iodine as it will dissolve. Chlorzinc iodine is the simplest reagent for cellulose, but acts much more rapidly in watery than in alcoholic sections, hence the latter should be placed in water. Glycerine sections can be placed in it, as the reagent diffuses very rapidly in glycerine.

— — *and iodine.* Dissolve iodine in chlorzinc iodine till a precipitate begins to be formed. This fluid stains the callus of sieve-plates a deep brown colour.

Chrom-acetic acid, 1 *per cent.* Chromic acid 0·7 per cent., acetic acid 0·3 per cent. in water. Used for "fixing" algæ. Time taken, up to twenty-four hours. *See* p. 248.

Chromic acid, 1 *per cent.* For fixing *Nitella*, filamentous algæ, etc. Twelve to twenty-four hours. After alcohol, the best of the simple fixing reagents, and preferable to it for many purposes.

— — 20 *per cent.* Used in preparing siliceous skeletons, pp. 96, 258.

— — 25 *per cent.* Dissolves membrane of pollen-grains.

— — *Concentrated.* Used as a macerating agent like Schultze's fluid, to which it is, however, inferior. Does not dissolve cork, 186.

(*All these Chromic Acid solutions are in Water.*)

Chromosmium acetic acid (Flemming's Fluid), prepared of varying strengths of chromic, osmic and acetic acids, and probably the finest of all fixing fluids. *See* pp. 248, 438 and 445.

Cloves, Oil of. Used for clearing sections prior to mounting in Canada balsam, etc. Preparations must be thoroughly dehydrated before using it.

Coaguline, for sticking card labels on micro-slides.

Cohn's normal solution, a culture fluid for certain bacteria, made of 1 gramme acid phosphate of potassium, 1 gramme sulphate of magnesia, 2

grammes neutral acetate of ammonia, and 0·1 gramme calcium chloride, dissolved in 200 c.c. distilled water.

Copper Acetate. Is a reagent for Tannin. For use, *see* p. 71.

— *Ammon-oxide* (cuprammonia, ammoniacal cupric oxide, also known as Schweizer's Reagent). Oxyhydrate of copper is carefully precipitated from a solution of the sulphate by a dilute solution of ammonia or of caustic soda; the clear green precipitate, separated and repeatedly washed with water, is added while still moist to strong ammonia, in which, upon slightly warming, it is dissolved. Upon cooling, crystals of sub-sulphate of copper and ammonia fall to the bottom. The filtered liquid contains only the ammoniacal cupric oxide in solution. It must be kept in bottles of dark glass, or in the dark. It can also be prepared by digesting copper turnings in an open bottle with the liquor ammon. of the Pharmacopœia. As it is very easily decomposed by light, it is perhaps best prepared fresh, when required. It is a reagent for cellulose, which it dissolves after great swelling. The early stages of swelling often bring out structure with great beauty. It is fit for use only so long as it rapidly dissolves cotton-wool.

— *Sulphate.* Used in Fehling's solution for sugar, 66.

Corallin solution. Dissolved in a 4 per cent. aqueous solution of carbonate of soda. To prevent its altering, a little camphor can be added to the solution. Constantly used for staining and differentiating mixed tissues. Specially stains lignin, sieve-callus and starch. According to Zimmermann, the callus reaction comes out best with overstaining and washing the surplus corallin out with 4 per cent. soda; and is especially good with fungi. The colour is not permanent.

Corrosive sublimate. Often used for fixing purposes. Can be removed from the preparation or fixed material by a solution of iodine.

Creosote. A good clearing reagent, but inferior to chloral hydrate for growing points, etc. It works very well after dehydration, as preparatory to mounting in Canada balsam.

Crystal Palace glass cement, or other similar cement, such as Coaguline. For fixing card labels on object-slides.

D.

Dammar, Gum. Dissolved in warm turpentine, and evaporated to the thickness of syrup. Can be obtained in tubes ready for use. It is a useful mountant for bacteria and filamentous algæ, after dehydrating and clearing, p. 302.

Diastatic ferment. Prepared as shown on p. 23 for the corrosion and solution of starch-grains.

Diphenylamine. 0·05 gramme in 10 c.c. pure sulphuric acid. Used as reagent for nitrates and nitrites. *See* p. 67.

E.

Eau de Javelle (Potassium hypochlorite). *Eau de Labarraque* (Sodium hypochlorite).

I give potassium hypochlorite the preference, though the two differ little in their action. *Eau de Javelle* can be prepared by stirring 20 parts of the officinal (25 per cent.) chloride of lime with 100 parts water, allowing it to stand some time, and adding a solution of 15 parts pure potash in 100 parts water. After allowing it to stand for one or several hours the mixture is filtered, and the filtrate used. Should lime still remain in the solution, and as a result the drops brought into use form in the air a skin of crystalline carbonate of lime, this is easy to remove by adding a few drops of potash solution and filtering off the precipitate. Used to delignify wood sections, and as a clearing reagent for growing points.

Egg, White of. Used, diluted with water, and with the addition of a little camphor, for observations in the embryology of Gymnosperms. *See* p. 374.

Ehrlich's Aniline-water fuchsin. To prepare and use, *see* p. 273. An admirable stain for Bacteria and their spores.

Enclosing (or mounting) Fluid for Algæ, in order to preserve the natural colour and form of Desmids, *Volvox*, and other Algæ, can be prepared by dissolving 1 gramme acetate of copper in a mixture of 130 grammes camphor water, 130 grammes distilled water, and 20 drops glacial acetic acid. To this is added 260 grammes glycerine, or, in cases, more or less, and the solution is filtered.

Enclosing (or mounting) fluid. Hoyer's, for Aniline preparations. A tall glass vessel with a wide neck is filled up to two-thirds with gum-arabic, in selected clear pieces. The vessel is then filled up to the neck with a solution of 50 per cent. acetate of potash, or with a watery solution of acetate of ammonia containing, to each 30 grammes, 10 grammes of caustic ammonia neutralised by a sufficient quantity of acetic acid. The gum is dissolved in a few days, if the vessel is often shaken, and forms a syrupy fluid, which is filtered through thick swansdown—a process taking about twenty-four hours.

Enclosing fluid for Carmine and Hæmatoxylin (Logwood) preparations. This is prepared as above, excepting that, instead of acetate of potash or of ammonia, a concentrated solution of chloral hydrate, to which is added 5 to 10 per cent. glycerine, is used. After some time this fluid may become turbid, and it is then necessary again to filter it.

Preparations mounted in either of these fluids require no further enclosing.

Eosin. Strong solution in alcohol. Stains protoplasm deeply. Especially useful for sieve-tubes, aleuron-grains with crystalloids, etc.

Eosin, Watery. Acts in much the same way. Also used to stain potassium nitrate solution in plasmolysis studies, pp. 46, 253.

— *logwood* (Rénaut's). Concentrated watery solution of eosin, 30 c.c., saturated alcoholic solution of hæmatoxylin, 40 c.c., glycerine saturated with potassium-alum, 130 c.c. The solution should remain unstoppered for some weeks till the alcohol has evaporated, and should then be filtered. A good double stain for nuclear structure.

Ether. As a reagent, used as a solvent for oils and ceric acid. Used also to dissolve cake celloidin. *See* p. 400. Also in the identification of Chitin in fungi, p. 239.

F.

Fehling's Solution. For preparation, *see* p. 66. A reagent for glucose and cane sugar.

Flemming's Solution or Fluid. See Chromosmium acetic acid.

Fuchsin. Dissolved in half-and-half alcohol and water. Sections treated previously with alcohol and stained with this, show up the structure of thickened cell-walls. Stained, and washed in absolute alcohol, the colour is removed from all excepting corky walls; more briefly washed, and controlled by careful watching, it is left also in lignified membranes; hence it can be used, *e.g.*, with logwood, as an excellent double stain.

— (*Acid*). Prepared by dissolving 0·2 per cent. acid fuchsin in distilled water, and a little camphor added to make the solution keep. A capital stain for chromatophores; but it must be remembered that it is soluble in alcohol, and this must not therefore be subsequently used.

— *iodine-green.* Prepared by pouring a solution of iodine-green in 50 per cent. alcohol into a saucer, and adding fuchsin, also dissolved in 50 per cent. alcohol, until the fluid takes a markedly violet colour. This stain is very useful in vegetable histology. The sections to be stained remain for about a minute in this solution, and are then transferred to glycerine. Excellent for nuclear staining after Flemming's fluid.

G.

Gelatine. Used as a nutrient medium in cultivation of pollen-grains, 391, and of bacteria, 283.

Gentian-violet. Very dilute watery solution, stains decolorised chlorophyll-bodies, and other chromatophores, bacteria, spermatozoids, and the nuclei of fungi.

— — *Acetic.* Gentian-violet is dissolved in 1 per cent. solution of acetic acid till the solution has a deep violet colour. A good nuclear stain.

These two stains are invaluable in preparations to show nuclear figures. The stained preparations keep in glycerine.

Gentian-violet. (*Gram's Method*). A mixture of 3 grammes aniline, 1 gramme gentian-violet, 15 grammes alcohol, and 100 grammes water. In nuclear staining (for which it is invaluable, especially after fixing by an acid method, such as picric, etc.), the sections remain in the stain from one to a few minutes, the stain is washed off with alcohol, and a solution of 1 part iodine and 2 parts potassium iodide in 300 parts water is at once added. This gives the sections a dark colour, and is at once washed off with alcohol; oil of cloves is added, which extracts more colouring matter from the sections, and usually first brings out the characteristic differential staining; finally the sections are mounted in Canada balsam. A very good double stain may be obtained by dissolving eosin in the clove oil. The walls and the achromatic nuclear figure appear pure red, and the chromatic figure violet (Zimmermann).

Glycerine. Used as a mounting fluid, also for clearing tissues by heating sections in it, and for dehydrating; also in differentiating corky from cuticularised membranes. *See* p. 186.

— *acidulated.* Used like acidulated alcohol (*q.v.*).

— *diluted.* Two parts glycerine, one part water.

— *and alcohol.* Half and half. Used especially for softening hard alcohol material.

Preparations showing fixed cell-contents are best not transferred direct from alcohol to glycerine, but placed in a mixture of alcohol and glycerine, allowed slowly to concentrate by evaporation of the alcohol, and similarly treated before mounting in glycerine jelly.

— *gum.* Ten grammes gum-arabic, 10 c.c. water, 40 or 50 drops glycerine. Used for embedding small or loose objects in section cutting, 231 (leaves of moss), 389 (pollen-grains), 409 (small seeds).

— *jelly* (Kaiser's). One part by weight of finest French gelatine is softened for about two hours in six parts by weight of distilled water. To this is added 7 parts by weight of chemically pure glycerine, and to each 100 grammes of the mixture is added 1 gramme concentrated carbolic acid (Phenol). It is then warmed for from ten to fifteen minutes, while continually stirring, till all the flocculence which the addition of the carbolic acid caused has disappeared. While still warm, it is filtered through the finest cloth of spun-glass or glass-wool, which has been previously washed in distilled water, and laid in the funnel while still damp. Can be obtained of E. Kaiser, Berlin. For notes on method of use as a mounting medium, *see* p. 31.

Gold-size. Finest English. Used for closing micro-preparations. *See* p. 31 (Glycerine-jelly), 37, 113 and 442 (Canada balsam).

Gum-arabic. Use in making enclosing fluids, etc., and for embedding. *See also* Glycerine-gum.

— — 10 *per cent.* clear filtered solution. Used for slackening movements of spermatozoids, 359.

Gum-arabic. For adhesive use on glass. 100 grammes gum-arabic in 250 c.c. water, to which is added a solution of 2 grammes crystallised aluminium sulphate in 20 c.c. water.

H.

Hæmatin-ammonia. *See* Ammonia-hæmatin.

Hæmatoxylin (Logwood), Böhmer's. Dissolve 0·35 gramme hæmatoxylin in 10 grammes absolute alcohol. This will keep indefinitely. For use add this solution drop by drop to a second solution of 0·1 gramme alum in 30 grammes distilled water until a beautiful blue-violet colour is produced. Allow the mixture to stand for a few days, and filter before using. *See* p. 146.

— — Grenacher's. Prepare—

(1) Saturated solution of hæmatoxylin in absolute alcohol.

(2) Saturated solution of ammoniacal alum crystals in distilled water.

Take 4 c.c. of (1) and mix with 150 c.c. of (2). Allow it to stand in the light for a week, filter, and add to it 22 c.c. glycerine and 25 c.c. methylated alcohol. Before use it is best to allow it to stand for some time till any precipitate has time to settle.

These hæmatoxylin solutions stain best if old, and should be used very dilute, *i.e.*, a few drops in a watch-glass of water. Sections of alcohol material, etc., must previously be placed in water to prevent a precipitate being formed on the surface. All acids must be avoided, though ½ per cent. hydrochloric acid is useful in case of overstaining. Logwood is invaluable for staining cell-contents, nuclear figures, etc.

Hydrochloric acid, ½ per cent. in 70 per cent. alcohol. Used in restoring overstained carmine preparations. *See* p. 249.

— — *10 per cent. in water.* Sometimes used as a sterilising fluid to keep utensils in.

— — *concentrated.*

— — mixed with from three to five times its bulk of water can be used for maceration. For method, *see* p. 159.

I.

Iodine, in alcohol. Officinal tincture of iodine, diluted with alcohol to a dark sherry colour. Or iodine dissolved in alcohol to the same tint.

— *in glycerine.* Iodine dissolved in glycerine, and water added if desired to dilute it. When undiluted, can be used to show minute grains of starch by carefully heating the preparation in a drop.

— *in potassium iodide.* Take 5 c.g. iodine, 20 c.g. potassium iodide, and 15 c.c. distilled water.

— *and sulphuric acid* (colours cellulose blue). Best obtained with potassium-iodide iodine, and sulphuric acid diluted with half its volume of water, *i.e.*, 2 vols. acid to 1 vol. distilled water.

Iodine (*Arthur Meyer's*). Details of preparation found in footnote to p. 20.

A special use of iodine is found in Gram's *Method of Bacterial Staining. See Gentian-violet and also Corrosive Sublimate.*

Solutions of iodine should be kept in darkness, or else in coloured glass bottles. Or the stock can be kept in a dark cupboard, and that in use replaced at intervals as seems necessary. For very exact purposes iodine solutions should always be kept in the dark.

— *See also Chlorzinc iodine.*

— *green.* Watery solution. Use for membrane staining, and as a rapid nuclear stain.

— — *Acetic.* In 1 or 2 per cent. acetic acid, iodine-green is dissolved until the fluid appears a deep blue-green colour. A rapid means of fixing and staining nuclei.

Iron acetate.
— *perchloride solution.*
— *sulphate solution.*
} Used in tannin reactions, 71. A dilute watery solution, to which a drop of nitric acid may be added.

K.

Knop's Culture Fluid, for fresh water Algæ. For preparation and mode of use, *see* pp. 251-2.

L.

Lemon, Oil of. Used as a clearing reagent for pollen-grains, 388, 390.
Linseed oil. Used for diluting gold-size.
Logwood. See Hæmatoxylin.

M.

Macerating mixture (*Schultze's*). Several methods for using this important reagent have been suggested.

(1) Place in a wide test-tube some pieces of chlorate of potash, and pour over them sufficient strong nitric acid to completely cover them; then lay in the fluid longitudinal sections of the material and warm over a flame till gas is actively evolved. Allow it to work for a few minutes, then empty the whole into a dish of water, and carefully wash. Remove the sections with a glass rod into another vessel of water, and thence into water on an object slide; there they can be torn to pieces with needles.

(2) Put the sections in a tube with an equal bulk of chlorate of potash, cover with concentrated nitric acid, and proceed as above.

(3) Use 1 gramme chlorate of potash to 50 c.c. nitric acid, and proceed as above.

(4) 3 grains chlorate of potash and 2 drachms nitric acid (sp. gr. 1·10); keep the sections in this, cold, for a fortnight.

After carefully washing in alcohol, the preparations as above can be preserved in glycerine.

Magenta. See *Fuchsin.*

Magnesia sulphate. Used in Knop's culture fluid for Algæ; Cohn's normal solution and Pasteur's Fluid.

Marjoram, Oil of. Clearing reagent, before mounting in dammar or Canada balsam.

Methyl-blue (Löffler's), 100 c.cm. potash of strength 1 : 1000, and 30 c.cm. concentrated alcoholic solution of methyl-blue. This solution can be preserved for years in a well-closed bottle. For staining bacteria in the tissues, *see* p. 274.

— — (Methylene-blue). Watery solution. Preparations washed, after staining, in water, show the cell-wall coloured, and not the protoplasm. Sections of alcohol material must be washed in water before staining. Is also taken up by the tannins.

— *green.* Alcoholic solution, used for material in absolute alcohol. Stain for five to thirty minutes, wash in distilled water, and mount in glycerine. Stains nuclei only.

— — *Acetic.* See *Aniline-green, Acetic.*

— *violet.* A concentrated alcoholic solution should be kept. For use, it can be added drop by drop to a little distilled water till this is deeply coloured. This fluid is specially used for staining the pellicle (zooglœa) of bacteria. A portion of the pellicle is placed on an object-slide, and a drop or two of the methyl-violet placed thereon and allowed to remain till the stain appears sufficiently deep. A little experience is needed here to judge the strength of the fluid and depth of the staining. If this is too deep, the jelly in which the bacteria are embedded is also stained. After staining, wash the preparation with water, or, better, with a 10 per cent. solution of acetate of potash. After lying for half an hour in the air, the preparation can be mounted in Canada balsam; not in glycerine, as that dissolves out the colour. Or it can be mounted in a watery (10 per cent.) solution of acetate of potash.

— — *Watery solution.* Used as stain for chromatophores, 53; also as a stain for the protoplasmic connections in sieve-tubes, the colour being removed from the walls by lying for a few days in glycerine.

Millon's reagent. Dissolve metallic mercury in its weight of concentrated nitric acid, and dilute with an equal volume of distilled water. Plugge recommends dissolving 1 part by weight of metallic mercury in 2 parts of nitric acid of sp. gr. 1·42, and then diluting with twice its volume of water. This reagent ought to be prepared fresh, but is said to be restored to a useful state by a few drops of a solution of potassium nitrate. Swells cell-walls, and displays their lamination. Protein substances are disorganised, but, after standing for a while, take a characteristic brick-red colour; slightly warming hastens this. (This reaction appears to fail sometimes. The crystalloids of *Ricinus* retain their form even when heated nearly to boiling point.)

Molybdate of ammonia. Dissolved in a concentrated solution of ammonium chloride.

Mordants, for fixing stains; an account of the use of is found on p. 111.

Mounting fluid. *See Enclosing fluid*, Canada balsam, Gum dammar, Glycerine-jelly, Glycerine, etc.

N.

Nigrosine (Quality I. of Trommsdorf). Watery solution.

— *Picric.* To a saturated watery solution of picric acid, a small quantity of watery solution of nigrosine is added till the fluid appears a deep olive-green colour. Exceedingly good for unicellular and filamentous algæ, staining and fixing at the same time (twelve to twenty-four hours). Also as a double stain for lignified and unlignified membranes. *See* p. 111.

Nitric acid. Used in xantho-proteid reaction (*q.v.*), and for maceration.

O.

Olive oil. Sometimes used as a temporary mountant. *See* pp. 33, 52.

Orange G. A good nuclear dye. Used for nuclear figures in saturated aqueous solution.

Orcin. In alcoholic solution this is used as a reagent for inuline. *See* p. 69.

Osmic acid, 1 or 2 *per cent.* *Must* be kept in darkness, and in a well-closed bottle. Colours oil-drops brown. Instantaneously fixes living protoplasm, and hence serves in nuclear studies. In a mixture of 9 parts 0·25 per cent. chromic acid solution, and 1 part 1 per cent. osmic acid, filamentous algæ, *Nitella*, etc., can be at the same time hardened and stained. *See* also Flemming's Fluid (Chromosmium acetic acid), and footnote on p. 445 for decolorising after.

P.

Paraffin. Used as an embedding material for sectionising. *See* p. 445. Paraffins of melting points 45° C. and 52° C. respectively are required. For some purposes harder paraffins with melting point up to 58° are useful.

Pasteur's Fluid. *See* p. 289 for preparation.

Phenol. *See* Carbolic acid.

Phloroglucin. Alcoholic (or watery) solution, 1 to 5 per cent., or even so low as $\frac{1}{10}$ per cent. will do. Followed by hydrochloric acid, is the best reagent for lignin. *See* pp. 78 and 134. Phloroglucin is expensive, but a convenient substitute can be prepared from cherry-wood. *See* Cherry-wood extract.

Picric acid. Saturated watery solution. A good fixing fluid, but requires very careful washing out, especially before using logwood as a stain.

— — Can be dissolved in sea-water for fixing marine algæ.

— *alcohol.* Picric acid dissolved to saturation in 50 per cent. alcohol.

Picric aniline-blue. To a saturated watery solution of picric acid, about 4 per cent. of a saturated watery solution of aniline-blue is added, so that a deep blue-green fluid is produced.

— *nigrosine.* *See Nigrosine, Picric.*

Potash (Potassium hydrate). Concentrated watery solution. A test for suberin (p. 186). Suberised tissues placed in it become yellow; if warmed, the colour is much deeper; if boiled, the suberin is exuded in the form of yellowish drops.

— *Dilute watery solution,* 5 or 6 per cent. Specially used as a "clearing" reagent.

— *Acetate of.* Concentrated solution in water. Used as a mounting fluid. After covering with the cover-glass, the preparation must not be sealed for about twenty-four hours. The fluid does not crystallise. *See* p. 216.

— *Alcoholic* (Russow's). Rectified spirit is mixed with a concentrated watery solution of potassium hydrate till a slight precipitate is formed. The fluid is frequently shaken, and allowed to stand for twenty-four hours. The resulting weakly yellow fluid is poured off from the sediment, and for use must be diluted with half its volume of distilled water. For the ordinary purposes of dilute potash this is preferable to the watery solution.

All these potash reagents, especially the watery solutions, must be kept in well-stoppered bottles, and the stopper occasionally anointed with vaseline.

— *Bichromate of.* 10 per cent. solution in water. Reagent for tannin.

— *Chlorate of.* Constituent of Schultze's macerating mixture (*q.v.*).

— *Nitrate of (Saltpetre).* A constituent of Knop's culture-fluid for algæ, 251.

— — in 10 per cent. solution. An excellent plasmolysing reagent.

— *Phosphate of.* A constituent of Knop's and Pasteur's nutrient solutions (*q.v.*).

— — — (*Acid*). A constituent of Cohn's normal solution (*q.v.*).

Potassio-sodic tartrate. Used in Fehling's sugar reaction, 66.

Potassium permanganate. Used in the preparation of Diatoms, 259.

Purpurin (Delta). Sometimes used with "solid green" or with chrysoidin as a double stain, 112.

R.

Rosaniline (or Fuchsin) violet (Hanstein's).—Equal parts methyl-violet and fuchsin (magenta) mixed and dissolved in alcohol. Shows up stratification of cell-walls, and differentiates sections of stems, especially monocotyledons. Stains protoplasm bluish-violet; amyloid substances, nucleus, and gums different shades of red; resins, blue; tannin, foxy-red; cellulose, pale violet; lignin, reddish; bast-fibres, deep-red; sieve-tubes and bast parenchyma, hardly at all. This is also an excellent stain for chlorophyll bodies.

Ruthenium red.

S.

Safranin in alcohol (absolute). Saturated solution. Stains nucleus well in material hardened in alcohol, chromic acid, or picric acid. In the latter two cases the sections must first be washed in water, and then placed in a little of the safranin solution diluted with an equal quantity of distilled water. Leave in this from twelve to twenty-four hours. They can be examined from time to time in a drop of the same fluid to see how the staining is proceeding. Then wash in absolute alcohol till they cease to give off colour; place in oil of cloves or of marjoram, and then mount in dammar or Canada balsam.

— *in water.* Saturated solution, one of the best single stains for differentiating tissues, and permanent. *See* pp. 111-2. Its action is illustrated on the stem of *Lycopodium*, 177.

— — A very highly recommended safranin stain is prepared by mixing equal quantities of a saturated solution in alcohol and another saturated solution in water.

Saltpetre. See Potassium nitrate.

Schultze's macerating mixture. See Macerating mixture.

Schweizer's Reagent. See Copper Ammon-oxide.

Shellac. The clearest possible, dissolved in absolute alcohol to the thickness of syrup, and filtered. Sometimes used as a mounting medium.

Sodium chloride (table salt). Used in 5 or 10 per cent. solution as a plasmolysing agent.

Starch paste (from Potato starch). Used in 1 per cent. solution as a nutrient material in pollen-cultures, 392.

Sugar (*Cane*). For solutions. 2·5 per cent. can be used to observe living cells in (pp. 38, 434); 15-20 per cent. solution (or stronger) to induce plasmolysis; 2-4 per cent. to induce conjugation in *Spirogyra* (p. 292); and solutions of various strengths, with 1·5 per cent. gelatine added, serve as culture fluids for pollen-tubes (p. 391 *et seq.*).

Sulphuric acid, concentrated.

— — *dilute.* Equal volumes of sulphuric acid and water. Also 2 acid : 1 water.

— — 12 *per cent.* Used as a test for Bacteria, 270.

T.

Tannin solution (in water). Used for colouring crystalloids. *See* p. 36. Also as a mordant, 111.

Tartar emetic (antimonio-potassio tartrate). Also used as a mordant, 111.

Turpentine.

V.

Vaseline.

Vesuvin. Water solution. Used for staining bacteria, 220.

W.

Wax. Small wax candles or "vestas". For making wax-spots on micro-slides to protect objects against pressure. *See* p. 442.

X.

Xanthoproteid reaction, for protoplasm. With successive use of nitric acid and ammonia, protoplasm colours yellow.

Xylol. A clearing reagent for micro-sections, and a medium for Canada balsam.

Z.

Zinc chloride. Used as a mordant. *See* p. 111.

NOTES.

Acetic Methyl-green. In Behrens' "Tabellen," the formula for preparation of acetic methyl-green is given as methylgrün 2·5 gr., water 100 c.c., glacial acetic acid 1 c.c.

Iodine glycerine (anhydrous). According to the same authority iodine glycerine may be prepared by dissolving 0·2 gr. iodine in 10·0 c.c. pure glycerine. It is used for the identification of tunicin, dextrin, glycogen, amyloid bodies, protein bodies, alkaloids, gums, slimes, latex, etc.

Ruthenium red (ammoniacal ruthenium sesquichloride). A reagent for pectic mucilages, etc. *See* note on p. 433.

APPENDIX IV.

GENERAL NOTES ON METHODS, AND SELECTED REAGENTS.

THE following notes include the most useful of the reagents given in Appendix III., and those therefore which it is desirable should be included in a limited selection. They are classified according to their purpose or nature.

For alcohol and methylated spirit, see a note at the beginning of Appendix III.

MICRO-CHEMICAL REAGENTS.—These are used on account of certain effects (reactions) which they produce upon some constituent or other of the plant, and by which therefore the presence and nature of that constituent can be recognised. Naturally in micro-chemistry we are restricted to optical reactions, and hence these reagents always produce distinctive effects, either swelling, dissolving, colouring, evolution of gas, etc.

(1) *Iodine in alcohol.*

(2) — *in glycerine.* Can generally be made from (3) when required. *See* also note on p. 498.

(3) *Potassium-iodide iodine.*

(4) *Chlorzinc iodine.*

Acids.

(5) *Sulphuric acid (concentrated).*

(6) — — *(dilute).* Can be prepared from (5) as required by mixing 1 drop with 1 or 2 of water.

(7) *Hydrochloric acid (concentrated).*

(7A) — — ($\frac{1}{2}$ per cent. in 70 per cent. alcohol).

7A can be prepared when wanted by putting 1 drop strong acid in a watch-glass of 70 per cent. alcohol.

(8) *Nitric acid* (strong).

(9) *Acetic acid* (glacial).

(9A) — — (2 per cent.). Stock solution for fixing fluids.

(10) *Chromic acid* (saturated).

(10A) — — (25 per cent.). Other percentages can be made from this as required.

(10B) — — (1 per cent.). Stock solution for fixing fluids.

(11) *Osmic acid* (2 per cent.). Stock solution for fixing fluids. Keep in dark.

(12) *Picric acid.* Saturated watery solution.

Alkalies, etc.

(13) *Ammonia* (strong).

(14) *Fehling's solution*, kept as stock.

(15) *Caustic potash* (concentrated).

(16) — — (5 or 6 per cent.).

Inorganic Salts.

(17) *Perchloride, Acetate*, or *Sulphate of iron.*

(18) *Bichromate of potash* (10 per cent. watery solution).

(19) *Chlorate of potash* (crystals).

Organic bodies.

(20) *Alcohol* (absolute, or 90 per cent.).

(20A) *Methylated alcohol*, old style (miscible with alcohol).

(21) *Ether.*

(22) *Alkanet*, root or tincture.

(23) *Aniline chloride* (in alcohol).

(24) *Camphor.*

(25) *Carbolic acid* (phenol).

(26) *Gelatine.*

(27) *Phloroglucin*, or *Cherry-wood extract.*

(28) *Cane sugar.*

(29) *Turpentine.*

PRESERVING FLUIDS.—The best is *methylated alcohol* (old style), of which a good supply should be kept. The material to be preserved for future work should be completely covered. For work in nucleus or protoplasm, 95 per cent. *alcohol* should be used.

SOFTENING.—Alcohol material, especially if old, is often brittle, and stems, etc., are hard. Soften for twenty-four hours in—

(30) *Half-and-half glycerine and meth. alcohol.*

If it seems desirable, the alcohol can be allowed to slowly evaporate, and the glycerine to concentrate.

HARDENING.—Soft tissues can often be hardened for cutting by three days in methylated spirit.

FIXING THE CELL-CONTENTS.—For studies in nuclei or protoplasm it is necessary to fix the cell-contents of the material, while quite fresh, without allowing them to contract. The material must be in very small pieces, so as to be rapidly permeable, and the fluid, except in the case of osmic acid, must be one hundred times the bulk of the material. The best fixing fluids are:—

(20) *Absolute* (or at least 90 per cent.) *alcohol.*

(10c) *Chromic acid*, 1 per cent., or *chrom-acetic acid*, 1 per cent.

(31) *Osmic acid*, 2 per cent.

(32) *Chrom-osmium acetic acid*, of varying strengths, prepared as required.

(33) *Picric acid* (saturated watery).

In chromic acid, chrom-acetic acid, or picric acid of the above strengths

the material can lie for twenty-four hours; then be laid in distilled water, frequently changed, for any time up to twenty-four hours. If not required for use at once, instead of this, first wash in 50 per cent. alcohol, then transfer to 70 per cent., then 80 per cent., and finally to 95 per cent. alcohol, or strongest methylated spirit, changing this after twenty-four hours if it is then discoloured. In this they can be kept for use (*see* p. 445).

Most algæ are best fixed in 1 per cent. chromic acid, or in picric acid, mixed with an equal volume of alcohol, for the same reason that alcohol potash is often preferable to watery potash solution, *viz.*, to avoid undue swelling of the walls.

SECTION-CUTTING.—Various hints on this subject will be found scattered in the text; *see* "Sections" and "Embedding" in the General Index. Where the material to be cut is alcohol-material, keep the razor and surface wet with alcohol; otherwise use water or, if the material is hard, glycerine. The razors are best hollow-ground; but for cutting wood use a razor only slightly hollowed, or flat on one side. Keep the razors sharp.

Thin objects which have to be cut can be placed in glycerine-gum, between pieces of elder-pith or smooth cork; or even, if moderately hard, between pieces of soft wood, such as poplar or lime-wood.

Very small objects may be embedded, either in celloidin or glycerine-gum (see these headings in Index). For very minute objects, such as pollen-grains, gum alone, or with a very small proportion of glycerine, can be used. A layer of strong gum is placed on the end of a piece of elder-pith, and when set nearly firm the pollen-grains dropped on it, and then another drop of gum placed on the top.

For cutting with a microtome, *see* p. 453 *et seq.*

Sections when cut may be lifted from the razor with a camel-hair brush. If laid on flatly, they will not curl. With such a brush a section can likewise be turned over. *See* p. 27.

CLEARING.—The cell-contents are often opaque; and when it is desired especially to see the cell-walls, as, *e.g.*, in growing-points, the preparation must be cleared. Clearing reagents act by dissolving, or at least swelling, the cell-contents. The best of these are:—

(16) *Caustic Potash* (5 or 6 per cent. watery).

(16B) — — *Alcoholic.*

(34) *Chloral hydrate.*

(35) *Eau de Javelle.*

After treatment with chloral hydrate or watery potash, wash in water; after alcohol potash, in alcohol, and then mount in glycerine. Eau de Javelle is specially used for alcohol material. For method, *see* p. 210. After treatment, place in dilute alcoholic glycerine, and allow this slowly to concentrate.

Sections of alcohol-material which have been stained and are to be mounted in Canada balsam or dammar, must be cleared in another

way. First place them for a minute or two in alcohol of the strength of that used for staining, and then into absolute alcohol for another like period. Thence transfer to—

(36) *Oil of cloves*, or,

(37) *Turpentine and creosote* (4 : 1).

Sections stained with aniline dyes should be treated as above, but cleared in—

(38) *Oil of cedar.*

STAINING.—This may be done in a watch-glass, or, better still, in small white earthenware saucers, such as are sold with cheap paint-boxes, especially could those be obtained of larger size, say 1¾ or 2-in. in diameter. As a rule, the best staining is obtained by dilute solutions and long treatment. The section to be stained must be immersed in the staining fluid, and examined from time to time to judge progress.

The object of staining is to show up (differentiate) diversities of structure, either of the plant skeleton or of the cell-contents, each stain particularising some special feature.

For a limited list, selected from those in Appendix III., the best are :—

(39) *Aniline blue.*

(40) *Acetic aniline-green.*

(41) *Corallin.*

(42) *Fuchsin* (Magenta) in 50 per cent. alcohol.

(43) *Acetic gentian-violet.*

(44) *Methyl-violet in water.*

(45) *Safranin in water.*

(These are all aniline dyes, stain rapidly, and the preparations fade more or less if kept in the light.. Safranin is most permanent.)

(46) *Mayer's Alum-carmine* (Carmalum).

(47) *Grenacher's logwood* (best old).

In many cases two stains can be used concurrently ; such are :—

(48) *Fuchsin iodine-green.*

(49) *Eosin-logwood.*

(50) *Rosaniline violet.*

MOUNTING.—Preparations can be mounted in

Glycerine.

— *jelly.*

Hoyer's mounting fluid (for aniline preparations).

Acetate of potash.

Canada balsam in turpentine or xylol.

Dammar.

No preparations containing water or glycerine can be mounted in Canada balsam or dammar. Watery preparations must be first dehydrated in alcohol; glycerine preparations first soaked for some time in water, and then in alcohol; and either of them afterwards cleared with oil of cloves.

For mounting in glycerine-jelly or Hoyer's fluid, water or alcohol must previously be removed by laying for some time in glycerine.

Preparations in acetate of potash retain their chlorophyll.

CLOSING.—Preparations mounted in Glycerine-jelly, Hoyer's fluid, Canada balsam, or dammar do not need further closing. In all these cases it is however desirable to put one or two thin layers of—

Gold-size with a fine brush, over the junction of cover-glass and object-glass. This must not be done till the mounting-fluid has set firm; and one layer of gold-size should be dry before another is added.

Preparations in glycerine should be hermetically sealed with Canada balsam in turpentine, laid round the cover-glass thinly with a thin glass rod, and, when dry, topped with gold-size.

Preparations in acetate of potash should be closed with gold-size.

LABELLING.—Paper labels may be stuck on the object-slides with *pure gum* containing a trace of glycerine or of aluminium sulphate, as described in Appendix II. Card labels, on the other hand, with *glass cement*, such as Crystal Palace cement, coaguline, etc.

The following is a still more restricted list, answering nearly all practical purposes:—

MICRO-CHEMICAL REAGENTS.—Potassium-iodide iodine, Chlorzinc iodine Sulphuric acid (conc.), Hydrochloric acid (conc.), Nitric acid (strong), Acetic acid (2 per cent.), Chromic acid (25 per cent.), Ammonia (strong), Osmic acid (1 per cent.), Caustic Potash, Perchloride of iron, Chlorate of potash (crystals), Ether, Alcanna tincture, Carbolic acid, 10 per cent. Sodium chloride solution.

PRESERVING FLUID.—Methylated alcohol (old style).

FIXING AND HARDENING FLUIDS.—Absolute alcohol, Chromic acid, 1 per cent., Acetic acid, 2 per cent.

CLEARING FLUIDS.—Potash in alcohol, Eau de Javelle or Chloral hydrate, Oil of cloves.

STAINS.—Methyl-blue, Corallin, Acetic gentiana-violet, Mayer's Carmalum (alum carmine), Logwood, Safranin, Methyl-violet, Fuchsin iodine-green, Eosin-logwood, Rosaniline violet.

MOUNTING MEDIA.—Glycerine, Glycerine-jelly, Canada balsam, Gold-size, Gum, Acetate of Copper enclosing fluid for Algae.

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